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THE ROLE OF THE STAPEDIUS REFLEX IN POSTSTIMULATORY AUDITORY FATIGUE

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Abstract The human ear is very resistant against noise induced damage in the low frequency range. The aim of the present study was to investigate whether or not the stapedius reflex is of any importance for this resistance. Subjects with peripheral facial palsy (Bell's palsy) including unilateral stapedius muscle paralysis were exposed to several different levels of narrow band noise centered at 0.5 and 2.0 kHz. Temporary threshold shift (TTS) at 0.75 kHz was significantly higher in the affected than in the nonaffected ear after 0.5 kHz noise at and above 110 dB SPL. After the exposure with 2.0 kHz narrow band noise there was no difference in TTS at 3.0 kHz between the affected and nonaffected ear. It is concluded that the attenuation provided by the stapedius reflex reduces TTS after low frequency noise exposure. An implication is that the stapedius reflex also may have the function of protecting the ear from hearing damage caused by low frequency noise exposure.

A reduction in the sound transmission through the middle ear caused by the acoustic middle ear reflex has been measured with different methods in man and has been found to be up to about 20 dB, mainly in the low frequency range (Pichler & Bornschein, 1957, Neergaard et al., 1963, Borg, 1968, Cancura, 1970). The role of the stapedius reflex in the development of noise induced temporary and permanent reduction of the hearing function remains, however, largely unknown.

Fletcher (1962) showed that a tone which elicited a stapedius reflex could diminish the temporary threshold shift (TTS) after exposure to rifle-noise by about 10 dB. Lehnardt (1959) found that TTS maximum after exposure to broad band noise was at 4.0 kHz when the stapedius reflex functioned normally, but shifted to 3.0 kHz when the stapedius muscle was paralysed by a muscle relaxant. Several investigators have used stapedectomized patients to estimate the influence of the stapedius reflex on auditory fatigue. The poststimulatory fatigue in the stapedectomized ear was compared with that in the contralateral ear (Steffen et al., 1963) or with that in the ears of a group of persons with normal hearing (Steffen et al., 1963, Ferris, 1965, 1967, Mills & Lilly, 1971). The results varied between these studies and are inconclusive. The variability was considered as possibly due to the fact that the hearing threshold of the operated ear was not fully restored or to incipient otosclerosis in the contralateral ear (Ferris, 1965, 1967, Mills & Lilly, 1971).

Patients with unilateral facial palsy have been shown to be more sensitive to auditory fatigue (Perlman, 1938), to have an increased sensation of loudness (Jepsen, 1955), and phonophobia (Hansen, 1965) on the paralysed side.

Efforts have been made to correlate single properties of the stapedius reflex with the individual sensitivity to temporary auditory fatigue. Johansson et al. (1967) found a close correlation between TTS at 0.5 and 1.0 kHz and the latency time, the rise time, and full activation time of the contralateral reflex in 5 subjects. Brasher et al. (1969), however, could not establish any significant correlation between TTS at 1.0 and 4.0 kHz and the reflex threshold, the contraction strength initially and after 2 min, of the contralateral reflex in normally hearing subjects.

This study was supported by grants from the Swedish Medical Research Council, the Medical Faculty, University of Umeå, P. Frenckner's fund and B. Fromm's fund

To summarize, it is known that the stapedius reflex attenuates the transmission through the middle ear of low frequency sounds It is not known, however, in what way or to what degree this affects auditory fatigue, nor is it known whether any reflex property can be correlated with the individual susceptibility to auditory fatigue (TTS) or with permanent noise damage to the ear These unknowns could preferably be investigated in patients with Bell s palsy and unilateral stapedius muscle paralysis Findings in the ear with stapedius paralysis can be compared with those of the same ear after recovery of the stapedius function and with the contralateral ear of the same subject Bell s palsy is fairly common and is sometimes found in young persons and thus it is possible to find subjects with normal hearing for the experiments

The aims of the present study were

(1) To determine the role of the stapedius reflex in development of auditory fatigue from exposure to sound at low (0 5 kHz) and higher (2 0 kHz) frequencies Preliminary results have been previously reported (Zakrisson & Borg 1974)

(2) To correlate the amount of the said fatigue ith the properties of the ipsilateral stapedius reflex

MATERIAL AND METHODS

The present study was performed in 22 subjects selected from a large group of patients with peripheral facial paralysis (Bell s palsy) of recent origin The selected subjects fulfilled the following criteria

(1) Hearing thresholds in both ears within 15 dB according to ISO Standard (1964) in the frequency range from 0 125 to at least 3 0 kHz The requirements for those to be exposed to high frequency noise were hearing thresholds within 15 dB ISO from 0 125 to 4 0 kHz

(2) Normal ear drums on otomicroscopic inspection

(3) No previous neurological disease or ear disorder as judged from the patient s case history

(4) No herpetic skin eruptions on the exte nal ear or in the ear canal

(5) Normal electro-nystagmogram during ca loric stimulation

The average age of the accepted patients wa 33 years (range 12–57 years)

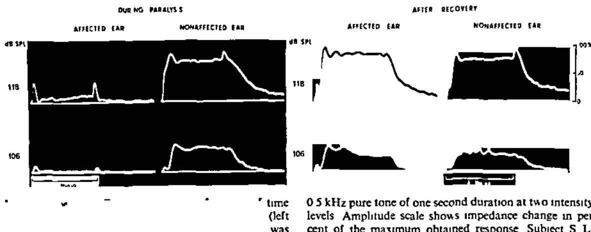
In 18 out of the 22 patients the Bell s pal y also included a stapedius paralysis in one e (the affected ear) They are called Experimental Group I in the subsequent presentation These subjects were characterized by the following stapedius reflex properties measured with the acoustic impedance change method (Moller 1961)

(1) Complete loss of the stapedius reflex in the affected ear upon contralateral stimulation with pure tones 0 5 kHz and 2 0 kHz up to 130 dB SPL Upon ipsilateral stimulation 6 of the patients had a slight reflex activity with a high threshold and low amplitude (see for instance the subject in Fig 1) The other 12 patients had a complete loss of the reflex in the affected ear upon ipsilateral stimulation as well

(2) In the nonaffected ear the ipsilateral reflex threshold (which was defined as the sound level that evoked 10% of the maximum obtained impedance change in that ear) was within 97 ± 10 dB SPL (mean ± 2 S D , see Borg & Zakrisson 1974) upon 0 5 kHz pure tone stimulation

The impedance change signals at 800 Hz were simultaneously recorded from each ear on a two-channel tape recorder During playback t signal was rectified and low pass filtered (32 H 18 dB per octave)

The acoustic stapedius reflex function r covered completely in 8 out of the 18 patier with unilateral stapedius muscle paralysis Th criterion of recovery was based on the degr of symmetry of the stapedius reflex respons between left and right ear In a contr l grou of 32 subjects Borg & Zakrisson (1974) calculat symmetry index It was found that 90 of th control subjects had an index below 30 In th present study the recovery of the stapedius fun tion was regarded to be complete when symmetr index was less than 30 Ipsilateral reflex record



time (left was

0 5 kHz pure tone of one second duration at two intensity levels Amplitude scale shows impedance change in per cent of the maximum obtained response Subject S L

ings in the affected and the nonaffected ear during paralysis and after recovery in one subject are shown in Fig 1 Only a very slight ipsilateral reflex response is seen in the affected ear during paralysis After the recovery reflex responses of the affected ear are very similar to those of the nonaffected ear In the nonaffected ear the reflex recordings equal each other on the two measuring occasions

Four out of the 22 patients with Bell's palsy had a symmetry index of less than 30 during acute paralysis, which qualified as a criterion for normal stapedius function These subjects are called Experimental Group II in the subsequent presentation

Experimental procedure

The experiments were carried out in a sound proof room with background noise less than 10 dB SPL within the frequency range 0 315–8 0 kHz (Bruel & Kjær ters filter) Auditory fatigue was measured as the TTS after noise exposure The pre-exposure threshold was determined with a Bekesy audiometer (Grason Stadler type E 800) during 1–2 min after which the noise exposure started and the audiometer was turned off The audiometer was turned on again at the same time as the noise was switched off and the post exposure threshold determined during at least 3 min Two exposure frequencies, 0 5 and 2 0 kHz, were chosen since it has been shown that the stapedius reflex attenuates sound effectively at 0 5 kHz whereas it has no, or only a slight, effect at 2 0 kHz (Borg 1968) One or several intensity levels were used with one exposure of each ear at each session A minimum period of time of one day elapsed between each session, allowing total recovery of the hearing thresholds to the pre exposure level

Sound exposure

Auditory fatigue was induced by

1) Bandpass filtered steady-state noise with a center frequency of 0 5 kHz and a bandwidth of 0 3 kHz at 3 dB points (Bruel & Kjær, type 1024, filter) The RMS level was varied from 90 to 120 dB SPL The exposure time was 5 or 7 min

2) Bandpass filtered steady state noise with a center frequency of 2 0 kHz and a bandwidth of 0 3 kHz The RMS level was 110 dB SPL The exposure time was 2 or 3 min

Calibration

The test tone from the Bekesy audiometer and the exposure noise were presented to the subjects through the same earphones (TDH 39 MX 41 AR Cushion) Calibrations of the earphones were carried out in a Bruel & Kjær 6 cc coupler and a Bruel & Kjær condenser microphone type 4132

Measurement of Temporary Threshold Shift (TTS)

It is known that the TTS maximum appears half an octave above the exposure frequency at pure tone (Theilgaard, 1949, Davis et al , 1950) or

To summarize, it is known that the stapedius reflex attenuates the transmission through the middle ear of low frequency sounds It is not known, however, in what way or to what degree this affects auditory fatigue, nor is it known whether any reflex property can be correlated with the individual susceptibility to auditory fatigue (TTS) or with permanent noise damage to the ear These unknowns could preferably be investigated in patients with Bell's palsy and unilateral stapedius muscle paralysis Findings in the ear with stapedius paralysis can be compared with those of the same ear after recovery of the stapedius function and with the contralateral ear of the same subject Bell's palsy is fairly common and is sometimes found in young persons and thus it is possible to find subjects with normal hearing for the experiments

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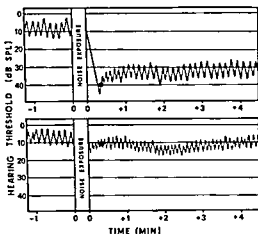
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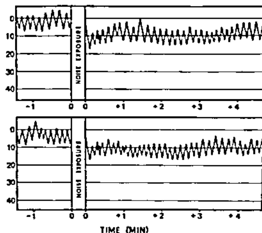


Fig 3 Bekésy tracings of the hearing thresholds at 0 75 kHz before (negative side of the time scales) and after (positive side of the time scales) exposure to 0 5 kHz noise 115 dB SPL 5 min in affected (upper graphs) and nonaffected ear (lower graphs) Left graphs During paralysis Right graphs After recovery The Bekesy audiometer was stopped during the time the ear was exposed to the noise ●, threshold values 20 sec after the end of the noise exposure Subject S L

the TTS, just as in the subject in Fig 3 The smaller amplitude in the Bekésy tracings was therefore probably a result of an increased attention and not an indication of recruitment of loudness

Ten of the subjects of Experimental Group I were exposed to 0 5 kHz noise at more than one intensity level Fig 4 shows individual TTS values for each subject (symbols) as a function of sound pressure level of the exposure noise in the affected ear It is seen that the TTS has a threshold around the lowest intensity tested in most subjects, except for the subjects GF and BM who were tested also well below threshold The TTS grows approximately as a linear function of the exposure noise level Such a linear relation has been shown in several earlier studies, e g Ward et al (1959) From the great individual variation in the position and inclination of the curves in Fig 4, it is seen that the noise intensity required to give a 10 dB TTS varies throughout a range of nearly 15 dB for the different subjects

Fig 5 shows means and S E M s for TTS 20 sec after the end of the noise exposure in the affected ear (continuous line) and in the nonaffected ear (broken line) during paralysis for Experimental Group I The difference in TTS between the affected and the nonaffected ear became manifest at the level of 105 dB SPL where it averaged 4 dB The difference was significant ($p < 0.001$) for noise intensity levels of 110 dB SPL and above It is also evident that there is

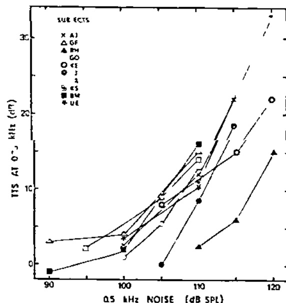


Fig 4 Individual TTS-values in the affected ear during stapedius muscle paralysis as a function of the intensity of the exposure noise in 10 subjects of Experimental Group I Exposure duration 5 min

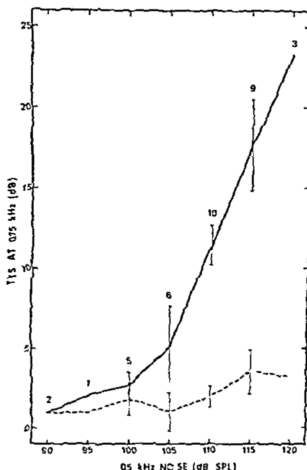


Fig 5 Mean values and S E M s for TTS during paralysis function of the intensity of the exposure noise ——, affected ears the number of values at each separate intensity are indicated ---- nonaffected ears Vertical bars *Standard errors of the mean Exposure duration* 5 min 18 subjects (Experimental Group I)

only a slight TTS in the nonaffected ear within the whole range of noise intensity levels used

The difference in TTS between the affected and the nonaffected ear measured 2 min after the end of the noise exposure was significant ($p<0.01$) after noise exposure of 110 and 115 dB SPL

The individual *TTS values in the affected ear* measured *2* min and *20* sec respectively after exposure correlated significantly at the levels of 110 and 115 dB SPL ($p<0.05$ and 0 01 respectively)

In the 4 subjects of Experimental Group II the difference in TTS between the ear with Bell s palsy and the other ear was 2 5, 0, −1 5 and −3 *respectively after* exposure to noise at 120 dB SPL during 5 min The average difference (−0 5 dB) was thus close to zero

Fig 6 shows TTS as a function of the *noise* intensity in *dB* in relation to the individual threshold of the stapedius reflex The thin lines with symbols show individual TTS values in the *affected ear during paralysis in 9 of the 10 subjects* shown in Fig 4 Subject U E was excluded due to error of calibration of the reflex stimulation tone A regression line was calculated for each individual series of TTS values The *heavy continuous line is based on the mean for* the inclinations and positions of these lines Similarly the heavy broken line was obtained from the individual TTS values in the non affected ear Since threshold values of the stapedius reflex at 0 5 kHz noise were not deter

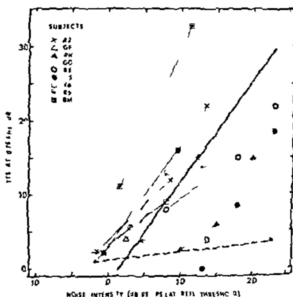


Fig 6 —— *individual* TTS values *during* stapedius *muscle* paralysis *as a function of* the exposure noise intensity re ipsilateral reflex threshold for 0 5 kHz pure tone Subjects symbols and exposure duration are the same as in Fig 4 The thick continuous line shows the mean slope and position of the regression lines from the individual results in the affected ear and the thick interrupted line shows corresponding details in the non affected ear *D* Difference between the noise intensity *which caused the same* TTS *in the affected* as in the non affected ear

Table I *Values of TTS and TTS-differences from exposure to 0 5 kHz noise in 8 subjects who recovered from their stapedius muscle paralysis*

TTS at 0 75 kHz (dB)
The exposure sound energy varied from subject to subject but was the same during paralysis and after recovery (intensity levels were 110, 115 or 120 dB SPL and exposure duration 5 or 7 min)

Subjects	K S	K H	S L	E L	K E	I A	R H	I J
During paralysis								
Affected ear (*a*)	33	22	32	18	22	14	15	29
Nonaffected ear (*b*)	7	4	3	5	2 5	3	0	4
After recovery								
Affected ear (*c*)	4 5	9	8 5	6	1	8 5	4	0 5
Nonaffected ear (*d*)	3	5	8	5 5	4 5	9 5	3 5	5 5
Differences								
c–d	Mean 0 3	Not significant						
a–c	Mean 17 9	Significant ($p<0\,001$)						

mined in all subjects, the thresholds for 0 5 kHz pure tone are indicated

The individual TTS susceptibility of the affected ear varied strongly in relation to the reflex threshold When the regression line of the individual series of TTS values was extrapolated a threshold of the TTS was obtained The lowest value for the TTS threshold was 5 dB below the reflex threshold (subject I A)

The threshold of the average difference in TTS between the affected and the nonaffected ear was about 2 dB above the ipsilateral threshold measured with pure tone (the intersection of the heavy lines) The mean for the ipsilateral stapedius reflex threshold at 0 5 kHz noise in the 7 patients in whom it was measured was 5 5 dB lower than the threshold at 0 5 kHz pure tone This relation between noise and pure tone is consistent with the results of Flottorp et al (1971), obtained in a larger group Thus the threshold of the difference in TTS is on the average about 8 dB above the ipsilateral reflex threshold for the 0 5 kHz exposure noise

Five of the subjects (A J, G O, I A, K S and B M) whose TTS thresholds were below the reflex threshold at pure tone had reflex thresholds at the 0 5 kHz exposure noise which were 5, 11, 5, 5 and 4 5 dB respectively below the corresponding thresholds at pure tone Thus also in these subjects the TTS thresholds were above the reflex thresholds for the exposure noise

In conclusion the difference in TTS between the affected and the nonaffected ear appears only in the range of noise levels where the stapedius reflex is active

It also follows from Fig 6 that a considerably higher noise intensity level is needed to obtain the same TTS values in the nonaffected ear as in the affected ear The maximum difference (*D*) was 19 dB However, this value includes some degree of uncertainty because it is based on small TTS values in the nonaffected ears

TTS measurements were repeated in 8 subjects of Experimental Group I who recovered from their stapedius muscle paralysis according to the criteria laid down by Borg & Zakrisson (1974) They were exposed to noise with an intensity and duration equal to that which produced the greatest TTS during the acute stage of the paralysis Table I shows that the TTS which was very pronounced during paralysis was significantly reduced ($p<0\,001$) when the stapedius activity had returned to normal in the affected ear (difference *a–c*) Upon recovery the affected ear was equally resistant to auditory fatigue as the normal ear (difference *c–d*)

In addition to the 8 subjects who have been reported in Table I, the TTS measurement was

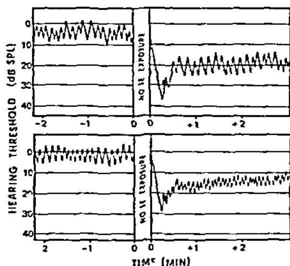


Fig 7 Bekesy tracings of hearing thresholds at 3 0 kHz before (negative side of the time scales) and after (positive side of the time scales) exposure to 2.0 kHz noise 110 dB SPL 2 min on affected (upper graph) and non affected ear (lower graph) ●, threshold values 20 sec after the end of the noise exposure Subject A J

repeated in the nonaffected ear in 4 subjects who did not fulfil the criteria for complete recovery f stapedius reflex function In all 12 subjects average TTS in the nonaffected ear was 1 dB ter on the second occasion No difference as greater than 6 5 dB

Exposure frequency 2 0 kHz

Fig. 7 illustrates the influence of the 2.0 kHz noise on the hearing thresholds in a representative subject during unilateral stapedius muscle paralysis The upper curve shows Bekesy tracing at 3 0 kHz in the affected ear and the lower curve shows the tracing in the nonaffected ear The patient did not experience any difference in loudness of the noise between the ears during exposure For the total number of patients (8) in whom identical exposure with 2 0 kHz noise was performed in both ears the average TTS at 3 0 kHz was 14 3 dB in the affected ear and 16 0 dB in the nonaffected ear There was thus a slight tendency towards a greater TTS in the nonaffected ear but the difference was not significant

DISCUSSION

The temporary threshold shift (TTS) after exposure to low frequency (0 5 kHz) and higher frequency (2 0 kHz) noise was measured in subjects with normal hearing and with unilateral Bell palsy involving paralysis of the stapedius muscle The results showed that the TTS after exposure to 0 5 kHz noise was significantly larger during stapedius paralysis than in the state of normal stapedius reflex after exposure to sound levels above the reflex threshold TTS after 2.0 kHz exposure was unaffected by the stapedius paralysis The findings can be due to the absence of attenuation of the stapedius muscle or to some other pathologic process related to the Bell s palsy and directly affecting the cochlea or the auditory nerve It is, however, concluded on the basis of the following facts that the increased susceptibility to auditory fatigue at low frequency exposure during stapedius paralysis was due solely to lack of the attenuating effect of the stapedius muscle contraction

1 The increased susceptibility to noise was only seen at low frequency Pathologic processes in the cochlea and the auditory nerve ought to influence a wide range of frequencies

2. Differences in TTS after low frequency exposure were seen only at sound levels above the stapedius reflex threshold (Fig. 6)

3 The increase in a given sound level that was necessary to apply to the normal ear in order to obtain the same TTS as in the ear with stapedius paralysis was about the same as the attenuation provided by the stapedius reflex (Fig. 6 and Borg & Zakrisson, 1974)

4 TTS after exposure to a low frequency noise was the same in both ears of the subjects in Experimental Group II

5 TTS after low frequency exposure decreased to normal values after recovery of the stapedius function (Table I)

6 The hearing thresholds were equal in the two ears up to 8 0 kHz during the acute stage of the Bell s palsy (Fig. 2) This was in contrast to earlier observations by Frank (1943) and Tsukamoto et al (1942) who found a slight

elevation of the hearing thresholds in the affected ear in patients with facial paralysis.

Is there any single property of the stapedius reflex that is correlated to the individual TTS after low frequency noise exposure?

The Bell's palsy subjects used in this investigation were tested at exposure frequencies and intensity levels which varied from individual to individual, thus the materials available for correlations were small. The rank-order correlation coefficients were nearly zero for the following relationships:

1. Ipsilateral stapedius reflex threshold to TTS in the affected ear during paralysis after 0.5 kHz noise exposure (at 110, 115 and 120 dB SPL),

2. The attenuation provided by the stapedius reflex at 0.5 kHz at 110, 115 and 120 dB SPL to TTS in the affected ear during paralysis at the same exposure intensity levels.

It thus seems as though the ipsilateral reflex properties were not correlated to the degree of auditory fatigue of the ear that is devoid of stapedius reflex. Neither was there any correlation between the ipsilateral stapedius reflex threshold and the TTS of the normal ear. However, there was a tendency to negative rank order correlation between the attenuation provided by the reflex at a certain sound level and the TTS of the nonaffected ear after 0.5 kHz noise exposure at the same level (rank order correlation coefficients −0.8 at 110 dB SPL, 0.04 at 115 dB SPL and −1.0 at 120 dB SPL). Negative correlation in this case means that subjects with a reflex that effectively attenuates the low frequency sound also have a small TTS value after low frequency exposure.

Implications for noise induced permanent threshold shift

A positive individual correlation between TTS and PTS (permanent threshold shift) at 1.0, 2.0, 3.0 and 4.0 kHz for exposure to continuous noise above 93 dB SPL has been demonstrated (Nixon et al., 1965). The present results show that the stapedius reflex reduces the TTS after low frequency noise exposure. The correlation between TTS and PTS shown by Nixon et al. (1965) supports the hypothesis that the stapedius reflex can also reduce PTS as a result of low frequency noise exposure. However, if the stapedius muscle is to play any role for the PTS it must have stable contraction during prolonged low frequency sound stimulation. It is known (Johansson et al., 1967, Anderson et al., 1969, Tietze, 1969, Blom & Zakrisson, 1974) that the response is stable during low frequency stimulation at least for time periods on the order of tens of seconds. The stability during long-term noise exposure has not been investigated.

Low frequency noise can be especially harmful under the following conditions:

1. When the low frequency sound is repetitive and has most of its energy within the latency of the stapedius reflex.

2. When the stapedius reflex response is abnormally weak such as when influenced by alcohol or barbiturates (Borg & Møller, 1967).

The main conclusion of the present study is that the attenuation provided by the stapedius reflex reduces noise induced TTS in the low frequency region which most likely also means protection against permanent damage of the ear.

ZUSAMMENFASSUNG

Das menschliche Ohr ist gegen Larmschadigung im tieferen Frequenzbereich sehr resistent. Die Aufgabe der vorliegenden Arbeit war nun zu untersuchen, ob der Stapediusreflex fur diese Widerstandsfahigkeit von Bedeutung ist oder nicht. Es wurden daher Patienten mit peripherer Fazialislahmung (Bell's palsy) und gleichzeitiger einseitiger peripherer Stapediusmuskellahmung mit Schmalbandgerausch von 300 Hz Bandbreite belastet, und zwar lag die Mittenfrequenz dieses Gerausches einmal bei 0.5 kHz mit Pegeln im Bereiche von 90 bis 120 dB SPL und zum andern bei 2.0 kHz mit einem Pegel von 110 dB SPL. Fur Pegel von 110 dB SPL und hoher war fur das Geräusch mit 0.5 kHz Mittenfrequenz die zeitliche Schwellenanderung (temporary threshold shift, TTS) bei 0,75 kHz in dem angegriffenen Ohr signifikant grosser als in dem normalen Ohr. Nach Belastung mit dem Schmalbandgerausch mit 2.0 kHz Mittenfrequenz war dagegen bei 3.0 kHz kein Unterschied im TTS zwischen

dem angegriffenen und dem normalen Ohr zu vermerken Daraus ist zu schliessen, dass die durch den Stapediusreflex bewirkte Schalldämpfung den TTS nach Belastung mit niederfrequentem Geräusch vermindert, und daraus kann weiter gefolgert werden, dass der Stapediusreflex auch eine Schutzfunktion gegen Gehorschadigung durch niederfrequente Gerauschbelastung haben kann

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A QUANTITATIVE ANALYSIS OF THE AFFERENT INNERVATION OF THE ORGAN OF CORTI IN GUINEA PIG

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Abstract A quantitative analysis of the afferent innervation of the organ of Corti was made on normal and vestibular nerve-sectioned guinea pigs Section of the vestibular nerve at the internal auditory meatus provided an efficient means of eliminating the efferent innervation to the cochlea without significant loss of afferent fibres Nerve counts on normal and de-efferented animals revealed that about 10–15% of the cochlear afferent innervation supplies the outer hair cells The remaining 85–90% of afferent fibres innervate the inner hair cells As in cats, all tunnel spiral bundle fibres and upper tunnel crossing fibres were efferent to outer hair cells Since unmyelinated fibres in the osseous spiral bundle were not counted, quantitative analysis of the efferent innervation to inner hair cells could not be made However a significant loss of myelinated fibres in the osseous spiral lamina after vestibular nerve section confirms that many myelinated efferent fibres are present in this region

The innervation of the organ of Corti has been the subject of numerous investigations for decades The identification of a cochlear efferent innervation by Rasmussen (1946, 1953) added a new dimension to the earlier and fundamental work of Retzius (1884), Held (1926), and Lorente de Nó (1937) Kimura & Wersäll (1962), Smith & Rasmussen (1963), and Spoendlin & Gacek (1963) demonstrated degeneration of efferent fibres and nerve terminals in the organ of Corti in three different mammalian species after destruction of the olivo-cochlear bundle. In the latter study, the authors sectioned the olivo-cochlear bundle in cats in order to evaluate the pattern of afferent innervation to the inner and outer hair cells Based on this and subsequent work on complete VIII nerve sectioning in cats, Spoendlin (1969, 1971, 1972) determined that the afferent innervation to outer hair cells comprises only 5% of the total cochlear afferent supply to the organ of Corti

To date, comprehensive quantitative analysis of the afferent innervation to the respective cochlear hair cell regions has been performed only in cats The present study evaluates the relative afferent fibre densities in guinea pigs Vestibular nerve sectioning is used to provide an efficient and accurate method of distinguishing afferent from efferent fibres within the organ of Corti With this procedure both crossed and uncrossed fibres in the olivo-cochlear bundle are destroyed while afferent cochlear neurons remain intact

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MATERIALS AND METHODS

Animal selection

Histological observations were made on 15 young female guinea pigs (250–300 g) which had vestibular nerve section performed 1–5 weeks prior to sacrifice Four of these animals, surviving 1, 3, 4, and 5 weeks respectively, met the requirements of successful olivo-cochlear

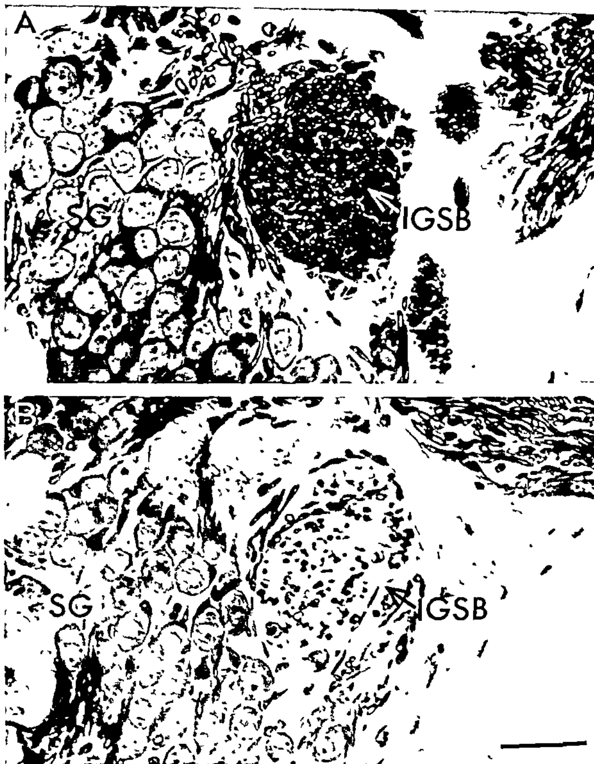


Fig 1 Radial sections of the spiral ganglion (*SG*) and intraganglionic spiral bundle (*IGSB*) from a guinea pig sacrificed five weeks after vestibular nerve section (A) Contralateral unoperated ear illustrating normal appearance of the spiral ganglion and intraganglionic sp[illegible] bundle (B) Vestibular nerve sectioned ear with degener[illegible] tion of the intraganglionic spiral bundle Bar at low[illegible] right equals 0 05 mm

blation and were included in this study The riteria of successful nerve sectioning were (1) normal histological appearance of spiral ganglion cells, (2) degeneration of fibres in the intraanglionic spiral bundle (Fig 1), (3) normal ppearing afferent hair cell synapses, and (4) bsence of efferent terminals in the organ of orti Three histologically normal unoperated emale guinea pigs (250–300 g) were similarly rocessed and analysed as normal controls

)livo-cochlear bundle ablation

n order to assure complete elimination of both rossed and uncrossed efferent fibres to the ochlea, the vestibular nerve was avulsed as it ntered the temporal bone Great care was taken ot to injure any cochlear afferent fibres Fortu ately there is a distinct separation between the ochlear and vestibular nerves at the level of the nternal auditory meatus in guinea pig and selecive sectioning of the vestibular nerve is feasible

Animals were anaesthetized with intraperitoeal injection of sodium pentobarbital (30 g/kg) and supplemented with local infiltration f 0 5 % lidocaine A mid line incision was perormed and the entire lateral surface of the skull rom the midcalvarium to the level of the exter al auditory meatus and from the nuchal crest o the root of the zygoma was exposed Bone as removed and the dura exposed over the ipsi teral cerebrum and cerebellum from the nuchal ne to the root of the zygoma The dura was ncised laterally near the neck of the paraocular lobe and the paraflocculus aspirated Vith the cerebellum retracted medially, the estibular, facial and cochlear nerves were readily entified under the operating microscope Jsing a fine stapes hook the vestibular nerve as avulsed from its foramen taking care not interrupt the cochlear nerve and blood supply hich lies beneath After nerve sectioning, the xposed dura was covered with absorbable elatin sponge (Spongostan) and the wound utured Sectioned animals were sacrificed from 5 weeks after surgery

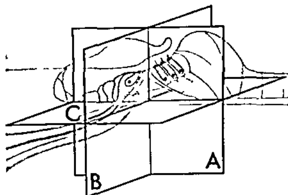


Fig 2 Diagram of the three planes of section used in this study (A) Radial (B) Tangential (longitudinal) (C) Horizontal

Cochlear preparations

The preparation of specimens for histological examination was the same in de efferented and normal animals The temporal bones from each guinea pig were removed immediately after sacrifice and the cochlea was locally perfused with cold 1 % osmium tetroxide through small openings created in the round and oval windows and in the cochlear apex The specimens were placed in 1 % osmium tetroxide 4 C for 2 hours, dehydrated in alcohol, and embedded in Epon Embedded cochleas were divided in the midmodiolus with a 0 3 mm thick circular saw Segments were taken from the first, second, and third cochlear coil and mounted for sectioning These cochlear segments were oriented and sectioned in three different planes to assist in a three-dimensional analysis of neural pathways (after Spoendlin, Fig 2)

1 *Radial sections*, perpendicular to the basilar membrane and cross sectional to the hair cells (standard cross sectional appearance of the organ of Corti), provided a cross sectional evaluation of the spiral ganglion and organ of Corti

2 *Tangential or longitudinal sections*, perpendicular to the osseous spiral lamina and parallel to the tunnel of Corti, were used to count fibres traversing the osseous spiral lamina and crossing the tunnel of Corti

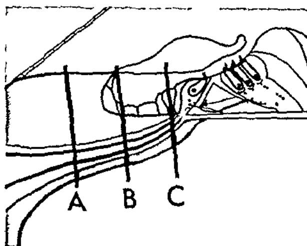


Fig 3 Tangential sections of the osseous spiral lamina were made in (A) presulcus (B) sulcus and (C) mid sulcus near the habenula perforata

3 *Horizontal sections*, parallel to the basilar partition, allowed observation of fibres travelling within the spiral bundles for distances of several pillar cells

All specimens were sectioned on an LKB ultrotome One micron sections were stained with toluidine blue for light microscopic examination and thin sections were stained with uranyl acetate d lead citrate for electron microscopy

erve fibre counts

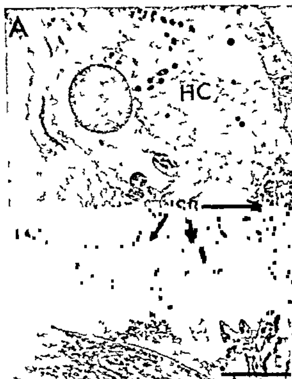
Counts of myelinated fibres within the osseous spiral lamina and unmyelinated tunnel crossing fibres in the organ of Corti were made in normal and nerve sectioned guinea pigs All counts were made from longitudinal photographic reconstructions of electron micrographs of tangentially sectioned specimens from the osseous spiral lamina and tunnel of Corti Distances were measured from a carbon line grid (21 600 lines/cm) which was photographed with the magnification and electron microscope settings unaltered Myelinated fibre counts from electron micrographs of the osseous spiral lamina were checked via light microscopy However, unmyelinated tunnel crossing fibres could not be accurately assessed with light microscopy and only electron micrographs were used in counting these fibres Fibre counts were made on 200–300 μm segments of the osseous spiral lamina and the tunnel of Corti in each of three cochlear coils Tangential sections were made from the area of the osseous spiral lamina through the tunnel of Corti to the outer hair cell region so that fibres counted in the lamina were related sequentially to those counted in the tunnel of Corti Laminar fibre counts were made in three different areas, pre sulcus, sulcus, and habenular regions to determine the extent of longitudinal spreading of radial fibres as they traverse the osseous spiral lamina (Fig 3)

RESULTS

Normal animals

Afferent nerve fibres innervating the organ of Corti travel through the osseous spiral lamina from the modiolus to the habenula perforata where these neurons lose their myelin sheaths. Efferent nerve fibres to the inner hair cell region pass from the habenula to the rather broad inner spiral bundle which lies at the base of the inner hair cells Afferent neurons pass through the inner spiral bundle to synapse with inner hair cells (Fig 4)

The afferent fibres to the outer hair cells travel from the habenula to a peripheral region of the inner spiral bundle which lies close to inner pillar cells Occasionally, an afferent fibre will pass directly from the habenula and enter the tunnel of Corti, but the majority of afferent fibres to the outer hair cell travel within the lateral aspect of inner spiral bundle passing one to three pillar cells before entering the tunnel (Fig 5) All outer hair cell afferent fibres pass between pillar cells to emerge beneath the tunnel spiral bundle Here, these fibres usually course downward toward the basilar partition As the inner pillar cell foot tapers toward the basilar membrane, the afferent fibres emerge into the fluid of the tunnel of Corti (Fig 6) In the tunnel they travel close to the surface of the outer pillar cell foot, rising to pass between the outer pillar cells before turning apically to become the lowermost fibres of the outer spiral bundle Afferent fibres travel within this bundle approximately 450 to 500 μm to synapse with outer hair cells





4 Normal inner hair cell region in guinea pig (A) adial section electron micrograph illustrat ng the rela onsh p of the tunnel sp ral bundle (*TSB*) and the inner piral bundle (*ISB*) to inner hair cells (*HC*) (B) Higher magn fication of the inner spiral bundle (*ISB*) coursing beneath inner hair cells (*HC*) The bars at the lower right corner equal 5 μm

g 5 Horizontal section through the inner hair cell in a normal guinea pig illustrating the passage of ent fibres (*Af*) between inner p llar cells (*PC*) This section is from an area below the tunnel spiral bundle The bar at the lower right equals 2 μm

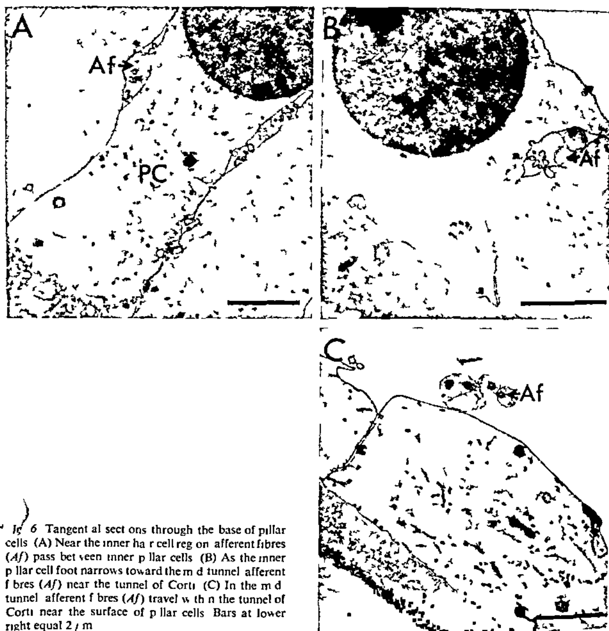


Fig 6 Tangential sections through the base of pillar cells (A) Near the inner hair cell region afferent fibres (*Af*) pass between inner pillar cells (B) As the inner pillar cell foot narrows toward the mid tunnel afferent fibres (*Af*) near the tunnel of Corti (C) In the mid tunnel afferent fibres (*Af*) travel within the tunnel of Corti near the surface of pillar cells Bars at lower right equal 2 μm

The efferent fibres to the outer hair cells enter the tunnel spiral bundle and travel usually from one to at least four pillar cells before turning outward to cross the tunnel of Corti (Fig 7) Efferent fibres cross the tunnel at a higher level than afferent neurons and tend to travel in groups of from 2 to 8 fibres These fibre groups pass between the outer pillar at about the level of the outer hair cell bases and in this region they appear to pass both apically and basally before terminating on hair cells The possibility of branching or *en passant* innervation cannot be excluded from the specimens studied

Vestibular nerve sectioned animals

Complete transection of the vestibular nerve in guinea pig resulted in destruction of the olivo-cochlear efferent innervation to the cochlea The loss of efferent fibres and terminals to the outer hair cells in the cochlea was complete within 5 weeks (Fig 8) Degeneration of efferent neurons within the tunnel spiral bundle was evident 1 week after nerve sectioning and was virtually complete by 3 weeks Interestingly in the 3 week

Table I *Myelinated fibre counts per mm in a normal and vestibular nerve sectioned animal*

Counts were made from tangential section in A Pre sulcus B Sulcus and C Mid-sulcus near the habenula perforata (see Fig 2)

	Normal 10 mm	Sectioned 10 mm	Sectioned 5 mm
A Pre-Sulcus	2 250	1 550	1 630
B Sulcus	1 810	1 280	1 340
C Mid-Sulcus	1 700	1 190	1 250

Table II *Average number of myelinated laminar fibres and unmyelinated upper (efferent) and lower (afferent) tunnel crossing fibres in three normal and three vestibular nerve sectioned guinea pigs*

Counts were made of fibres in the basal, middle and apical cochlear regions Counted cochlear segments were made 6–7 mm 10–11 mm, and 15–16 mm from the round window respectively

	Normal	Sectioned
Basal (6–7 mm)		
Lamina	1 765	1 231
Upper tunnel	570	0
Lower tunnel	125	120
Middle (10–11 mm)		
Lamina	1 713	1 157
Upper tunnel	418	0
Lower tunnel	155	148
Apical (15–16 mm)		
Lamina	1 583	1 124
Upper tunnel	235	0
Lower tunnel	115	118

survivor degenerating efferent nerve fibres could be seen in the tunnel spiral bundle of the first and second coils while in the third coil there was no trace of the tunnel spiral bundle (Fig 9) By 5 weeks all fibres in the tunnel spiral bundle had disappeared Similarly, all upper tunnel fibres degenerated after vestibular nerve sectioning and only lower tunnel crossing fibres remained (Fig 10)

In sectioned animals afferent fibres crossing the lower portion of the tunnel appeared normal as did the fibres within the outer spiral bundle The inner spiral bundle lost a great number of neurons especially in the region nearest the inner pillar cells (Fig 11) and similarly the osseous spiral lamina reflected the loss of myelinated efferent neurons

In both normal and vestibular nerve sectioned animals there was a reduction in the number of myelinated fibres within the osseous spiral lamina as the distance from the spiral ganglion increased (Table I) To assess relative afferent innervation densities to the cochlear hair cells, only laminar fibre counts in the habenular region were correlated with counts of upper and lower tunnel crossing fibres

Afferent fibres

The results of fibre counts in both normal and "de-efferentized' animals is summarized in Table II In the normal guinea pigs studied, about 6–9 % of all myelinated fibres in the osseous spiral lamina were afferent cochlear neurons to the outer hair cell region The remaining 91–94 % of myelinated laminar fibres comprised the afferent supply to the inner hair cell region as well as a portion of the cochlear efferent supply In animals in which the olivo-cochlear bundle was sectioned, all surviving myelinated fibres were cochlear afferents to the inner and outer hair cell regions Comparing the number of myelinated laminar fibres in de-efferentized animals with those in normal guinea pigs, it is possible to conclude (after subtraction of the outer hair cell afferents crossing the tunnel of Corti) that approximately 55–60 % of all myelinated laminar fibres in normal animals were afferent fibres innervating the inner hair cell region About 10–15 % of surviving myelinated laminar fibres in nerve sectioned animals were cochlear afferents to outer hair cells Thus the afferent innervation density to the inner hair cells was approximately six to nine times greater than to the outer hair cell region Interestingly, the afferent innervation densities to both inner and outer hair cell regions appeared relatively constant throughout the three cochlear segments quantitated

Efferent fibres

Myelinated efferent fibres appear to comprise about 25–30 % of all myelinated ' the

Fig 7 Horizontal section through the inner hair cell region at the level of the tunnel spiral bundle (*TSB*) Efferent fibres (*Ef*) pass between pillar cells to enter the tunnel spiral bundle Bar at lower right equals 2 μm

Fig 8 (A) Base of outer hair (*HC*) in normal guinea pig illustrating large vesiculated efferent (*Ef*) and afferent (*Af*) terminals (B) 3 weeks after vestibular nerve section Efferent terminals disappear and only afferent fibres (*Af*) remain Bars at lower right equal 1 μm

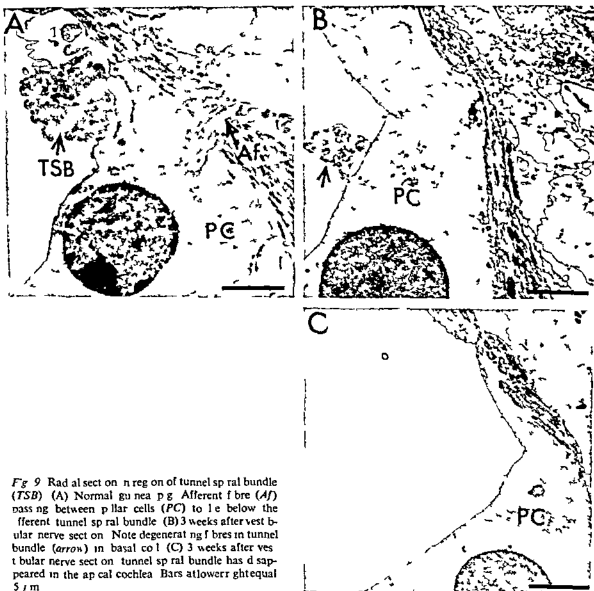


Fig 9 Radial section in region of tunnel spiral bundle (*TSB*) (A) Normal guinea pig Afferent fibre (*Af*) passing between pillar cells (*PC*) to lie below the efferent tunnel spiral bundle (B) 3 weeks after vestibular nerve section Note degenerating fibres in tunnel bundle (*arrow*) in basal coil (C) 3 weeks after vestibular nerve section tunnel spiral bundle has disappeared in the apical cochlea Bars at lower right equal 5 μm

osseous spiral lamina in guinea pig Since counts within the osseous spiral lamina were limited to myelinated fibres the total efferent supply to organ of Corti could not be determined owever outer hair cell efferent fibres were ysed as these fibres crossed the tunnel of Corti In normal guinea pigs outer hair cell crossed the tunnel of Corti at a higher level than their afferent counterparts Indeed were it not for this spatial separation within the middle of the tunnel of Corti it would be ex ly difficult to distinguish between outer cell afferent and efferent fibres

Fibre size is not useful in differentiating outer hair cell afferent and efferent fibres as is illustrated in Fig 12 The diameters of 100 upper tunnel (*efferent*) *fibres and 60 lower* (*afferent*) fibres are graphically represented Measurements were made from electronmicrographs of tangential sections through the middle of the tunnel in normal animals Note that there was a rather broad range in both afferent and efferent fibre size but that the diameters were rather similar for both nerve populations

The efferent nerve supply to the outer hair cells was considerably more extensi

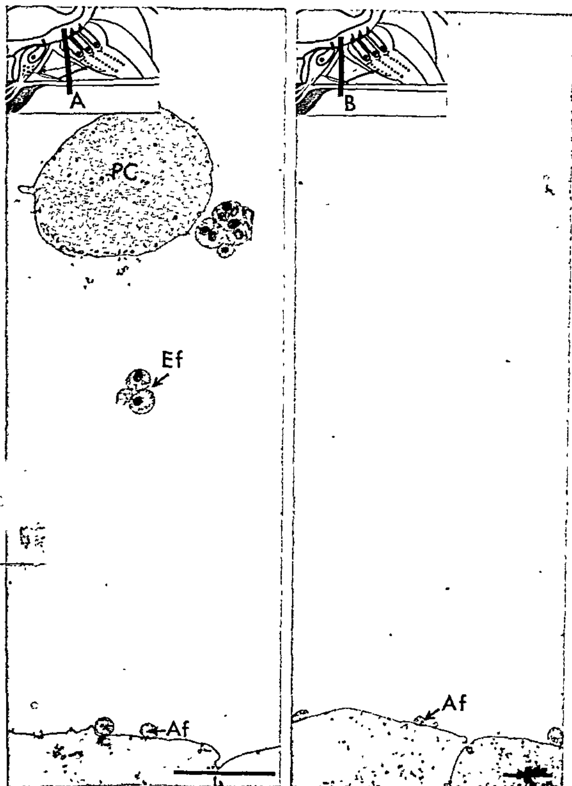


Fig. 10. (A) Normal animal tangential section through the outer pillar cell region of the tunnel of Corti (see middle of the tunnel of Corti (see inset diagram) 5 weeks after vestibular nerve section. Only lower tunnel afferent fibres remain, all upper tunnel crossing efferent fibres have degenerated Bars at lower right equal 2 μm.

Fig 11 Appearance of the inner hair cell region 5 weeks after nerve section afferent fibres (*Af*) remain but efferents in the inner spiral bundle have disappeared Bar at lower right equals 2 μm.

innervation Upper tunnel crossing fibres were about twice as numerous in the third coil as the lower tunnel crossing afferent fibres and in the basal coil they were approximately five times more numerous than outer hair cell afferent fibres (see Table II)

DISCUSSION

ecent work by Spoendlin (1971 1972) based on both olivo-cochlear bundle ablation and destruction of cochlear afferent and efferent ibres by total eighth nerve sectioning at the nternal auditory meatus in cats revealed that about 5% of cochlear afferent fibres cross the unnel of Corti to innervate outer hair cells The ning 95% of the afferent cochlear fibres ly the inner hair cell region He found that vation densities for both afferent and effer fibres in cat were greatest in the basal and iddle coils and lowest in the apical area The

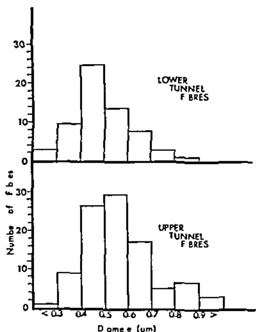


Fig 12 Graphic representation of the fibre diameters of 60 lower tunnel afferent fibres and 100 upper tunnel efferent fibres

number of tunnel crossing fibres was relatively constant throughout the cat cochlea, with the efferent fibres to the outer hair cells two to three times more numerous than their afferent counterparts

In the osseous spiral lamina of guinea pigs there are approximately 1765 myelinated fibres/mm in the basal turn (5–6 mm), 1713 myelinated fibres/mm in the second turn (10–12 mm) and 1585 myelinated fibres/mm in the third coil (15–16 mm) These figures are considerably less than those reported in cats but are similar to those reported by Spoendlin (1972) in guinea pig It is important to stress that these counts were made on myelinated fibres in the inner sulcus near the habenula perforata Unmyelinated sympathetics and efferent fibres were not counted in normal or sectioned animals

In the normal guinea pig there is a significant reduction in the number of myelinated fibres/mm when laminar counts made at the habenula region are compared to counts in the presulcus area We believe that this apparent reduction of fibre population is the result of longitudinal reading analogous to the radiating spokes of eel as they travel from the axle to the rim de were to count the spokes per metre near e axle, one would have a much larger number than counting the number of spokes per metre near the rim In the cochlea, our interest was to determining the proportion of myelinated radial nerve fibres which cross to the outer hair cell region, and therefore counts near the habenula were essential

As in the cat, the afferent innervation to the outer hair cells in guinea pigs is small representing approximately 10–15% of the total cochlear afferent innervation The inner hair cell region receives 85–90% of cochlear afferents traversing the osseous spiral lamina The efferent innervation to the outer hair cell region, comprising all upper tunnel crossing fibres, is approximately twice as dense as the afferent supply in the 15 mm cochlear region In the basal coil the outer hair cell efferent population is almost five times greater than their afferent counterparts These figures are also quite similar to those noted in cats

The hazards of interpreting the results of sectioning the olivo-cochlear bundle are well known Successfully performed vestibular nerve sectioning can be an efficient means of eliminating both crossed and uncrossed olivo-cochlear fibres Its major drawback is possible injury to the underlying cochlear nerve and potential interruption to the vascular supply to the cochlea

In the guinea pig selective avulsion of the vestibular nerves can be accomplished without apparent loss of the afferent supply to the cochlea The "de-efferentized" animals selected for inclusion in this study all had normal appearing spiral ganglion cell populations and afferent hair cell synapses

Terayama et al (1969, 1971) demonstrated in guinea pigs that section of the olivocochlear bundle as it crosses the floor of the fourth ventricle leads to degeneration of both myelinated and unmyelinated components of the bundle in the cochlea They were able to trace degenerating myelinated and unmyelinated fibres in centrally sectioned animals through the intraganglionic spiral bundle and into the osseous spiral lamina The loss of most myelinated fibres within the osseous spiral lamina after vestibular nerve sectioning in our series is likely the result of destruction of both crossed and uncrossed myelinated fibres of olivo-cochlear bundle and not necessarily the result of damage to the cochlear nerve In the guinea pig, myelinated efferent fibres appear to comprise a significant portion (25–30%) of the total myelinated fibre population traversing the osseous spiral lamina

The efferent supply to the organ of Corti was analysed only in relation to the efferent fibres which cross the tunnel of Corti to innervate outer hair cells Investigations are presently underway to quantitate both myelinated and unmyelinated efferent fibres as they travel from the osseous spiral lamina to their respective terminals in the organ of Corti

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ZUSAMMENFASSUNG

Eine quantitative Analyse der afferenten Innervation des Cortischen Organs bei normalen Meerschweinchen wurde nach Durchtrennung des N vestibularis durchgefuhrt Die Vestibularisdurchtrennung im inneren Gehorgang erwies sich als wirksames Mittel zur Ausschaltung der efferenten Innervation der Cochlea ohne signifikanten Verlust von afferenten Fasern Nervenzahlungen bei normalen und „ent-efferentisierten" Tieren ergaben, dass ungefähr 10–15 % der cochlearen afferenten Innervation die ausseren Haarzellen versorgen Die restlichen 85–90 % der afferenten Fasern innervieren die inneren Haarzellen

Wie bei der Katze waren alle Fasern des Tunnelspiralbundels und alle oberen Tunnel kreuzenden Fasern efferent zu den äusseren Haarzellen Da myelinfreie Fasern in dem knochernen Spiralbundel nicht gezahlt wurden, war eine quantitative Analyse der efferenten Innervation der inneren Haarzellen nicht moglich Ein signifikanter Schwund myelinhaltiger Fasern in der Lamina spiralis ossea nach Vestibularisnervdurchtrennung bekraftigt je doch die Tatsache, dass zahlreiche myelinhaltige efferente Fasern in diesem Gebiet vorkommen

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OBSERVATION OF CLICK-EVOKED COMPOUND VIII NERVE RESPONSES BEFORE, DURING, AND OVER SEVEN MONTHS AFTER KANAMYCIN TREATMENT IN THE GUINEA PIG[1]

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Abstract Compound VIII nerve action potential responses to clicks and filtered-clicks were recorded regularly over a period of 7 months following Kanamycin treatment (8 days, 400 mg/kg) in 3 awake guinea pigs each with an implanted electrode on the round window (left ear) From the end of the treatment the responses to the click became *dissociated* in various degrees within three major phases (1) a rapid phase (8 to 10 days) where the responses were dramatically altered, (2) a recovery phase which lasted about 2 months, (3) a slow phase over the quent 5 months where the responses to the click sed slowly but gradually while the N 1 component, l as the responses to the 8 000 Hz filtered-click h became *recruiting* at the end of the treatment), sappeared progressively Responses to the low frequency filtered-clicks were less affected Cochlear cytograms showed almost complete degeneration of the inner and outer hair cells of the first turn only

Although the ototoxicity of some antibiotics has been extensively studied, the mechanisms of this toxicity are still far from understood, and, up to the present time, only the slow process of accumulation of data obtained through different approaches can help to find an answer to the problems raised by this ototoxicity

Having developed a method which allows precise observation over a long period of time of the sound-elicited activity of the VIII nerve (through click-evoked VIII nerve compound action potential recordings), it seemed appropriate to apply this technique to the study of the development of changes produced by antibiotic intoxication, monitoring the activity of the nerve in animals treated with Kanamycin

Such chronic electrophysiological observations have been occasionally attempted, but over too short a period of time (Farkashidy et al, 1963) or they have been concerned only by the cochlear microphonic (Galambos, 1960, Ouchi & Ohtani, 1969, Simmons et al, 1960) In this study, attention has been focused on the compound VIII nerve action potential which is recorded in a manner very similar to that used in man during electrocochleography (Aran, 1971) Only the site of the active electrode is different (round window instead of promontory) but the signal processing, averaging, cancellation of microphonic potentials, stimuli and so on, are similar This was done because such animal experiments, together with final histopathological observations, are intended to verify and complement the interpretations proposed for the normal and pathological patterns of the same responses recorded in man (Aran, 1972, 1973). Since Kanamycin is well known as causing damage to the hair cells of the organ of Corti, starting with the external hair cells of the base

[1] This work is part of a Doctoral dissertation (Doctorat es Sciences) presented by J M Aran at the University of Bordeaux II (Thesis n° 335) on September 15 1973 "Analyse du Fonctionnement Global du Nerf Auditif Etude Electro-cochleographique normale et pathologique chez l homme et vérifications expérimentales chez l animal"

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of the cochlea (Hawkins, 1959, Hawkins et al, 1967), it seemed reasonable to use this in attempts to reproduce in the guinea pig the *dissociated* pattern of VIII nerve response observed in man This pattern is consistently recorded in patients presenting with sensorineural hearing loss of cochlear origin with the loss limited to the high frequencies, but with some residual hearing and recruitment at these frequencies Previous experiments using intense acoustic stimulation (white noise, high frequency pure tones) have succeeded in producing this *dissociated* pattern but failed in showing a relationship between this pattern and the pattern of hair cells damage in the cochlea as shown by phase contrast microscopy (Portmann et al, 1973)

MATERIALS AND METHODS

Electrode implantation

Guinea pigs are equipped with an electrode (nickel-chrome wire, 50 μm in diameter, bent in a loop) permanently implanted on the round window membrane The reference and ground electrodes were taken on the screws placed on the skull to hold the electric plug where the three electrodes are soldered The method of electrode implantation has been already described (Portmann et al, 1966) and is quite similar to the methods proposed by others (Buño et al, 1966, Farkashidy et al, 1963, Galambos, 1960, Ouchi & Ohtani, 1969, Simmons et al, 1960, Toyama et al, 1966) Special attention to the various stages of the procedure and good training are necessary to make such a technique easy and successful

Stimulus presentation

One problem in using awake and mobile animals is in delivering the acoustical stimuli to the ear drum This is quite difficult in free field conditions This problem has been carefully studied by Webster & Dunlop (1965) and it is obvious that movements considerably modify the intensity, the wave form and the travelling time of the acoustic stimulus from the speaker to the ear This is completely incompatible with the accurate recording of such fast and precise response as the VIII nerve action potential where the three parameters which are studied amplitude, latency, and wave form, are all dependent on the physical characteristics of the stimulating signal This problem was easily solved in the awake guinea pig by delivering the stimuli directly to the drum through a short plastic tube which is attached to the plug on the skull and which is bent so that the other end fits into the external acoustic meatus and is maintained there by the elastic pressure of the tube (Portmann et al, 1973, Fig 3) This method is much more simple than the method proposed by Buño et al (1966) who introduced the sound directly into the middle ear cavity The sound is directed from a TDH 39 Telephonics receiver to the plug on the skull through another plastic tube which travels with the wires connecting the electrodes to the preamplifier Thanks to the guinea pig's congenital equanimity, the tube is well accepted in the ear canal during the recording sessions and the measurements can be performed very easily, the activity of the guinea pig being monitored by listening to the signal from the electrodes The guinea pig is placed in a cage acoustically and electrically treated (Amplifon), itself placed into a shielded, sound-proof room (Amplifon, Type G, large)

Stimuli

The stimuli used are clicks produced by 0 1 ms rectangular electrical impulses sent into the receiver, and filtered-clicks produced by rectangular impulses filtered into a band-pass passive filter centred on the various test frequencies 8 000, 4 000, 2 000 and 1 000 Hz (band-width $\pm 10\%$ around Fo, attenuation 30 dB/octave) (Filter Allison 2AB) The width of the pulses is respectively 1/2 Fo The click intensity is measured in dB peak equivalent SPL For the different filtered-clicks the same level expressed in dB corresponds to the same amplitude of the rectangular impulse at the input of the filter, as for the click These stimuli are presented at a rate of 10/sec

Recording

The equipment here is also the same as that used for human electrocochleographic recordings

Pre amplifier—P A R CR4A (differential, input impedance 50 MΩ, ×1000, 3–30 kHz) Amplifier—B & K 2409 (×10, 2–200 kHz) Averager—P A R TDH9 (linear mode, prefiltered CTC 5 s ×10 or 1, window 10 ms but for the 1 000 Hz filtered click (20 ms)) Double trace oscilloscope CRC Rectilinear strip chart recorder TELCO ED 26

999 (near threshold) to 125 (at 130 dB) responses are added in the averager The microphonic potentials are cancelled by reversing the polarity of every other click The VIII nerve action potential is recorded from threshold to 130 dB in steps of 10 dB

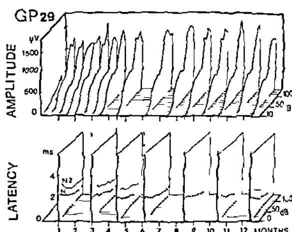


Fig 1 3D (without correction) representation of the input–output functions amplitude (peak to-peak) and latency (N1 and N2) versus click intensity of the VIII nerve compound action potential recorded over 12 months in normal guinea pig no 29 The thick line on the latency diagram joins the latencies of the larger peak in the responses (large dots) which in the normal guinea p g is always N1

Design of the experiment

Three guinea pigs with the implanted electrode on the round window of the left ear, showing normal responses over 1 month after the electrode implantation, were given intramuscular ections of Kanamycin on a 400 mg/kg basis, day for 8 days Responses to the clicks threshold to 130 dB were recorded every y for 23 days after the beginning of the treatment, then about once every week over 7 months Responses to the various filtered clicks were recorded before, 3 months after, and 7 months after the treatment The general condition of the guinea pigs was monitored by changes in their weight On the day of sacrifice, prior to osmium acid fixation of the cochlea responses of the right ears to the clicks and the filtered clicks were recorded under general anaesthesia During the same period, normal untreated guinea pigs with implanted electrodes were observed, as controls

RESULTS

Controls

Normal responses can be observed in implanted untreated guinea pigs for periods well over 7 months since several guinea pigs have been followed up for more than a year and still present normal responses (Fig 1) These guinea pigs are used for ongoing studies on the different characteristics of the normal responses

During the treatment

The responses were barely modified during the 8 days of treatment A slight increase in the maximum amplitude could be observed during the final days However, at this time the weight of the guinea pigs started decreasing

After the treatment

The results were absolutely identical in the 3 guinea pigs (Fig 2) and are presented in detail for one of them (GP no 37)

The changes in the responses can be divided into three major phases (1) a rapid phase (8 to 10 days) where the responses are dramatically altered (2) a recovery phase which lasted for about 2 months (3) a slow phase over the 5 subsequent months where the responses decreased very slowly but inexorably

During these changes, that is from the end of the treatment, the weight of the guinea pigs increased steadily and, at the end of the experiment was almost twice what it was when the electrodes were implanted (Fig 3)

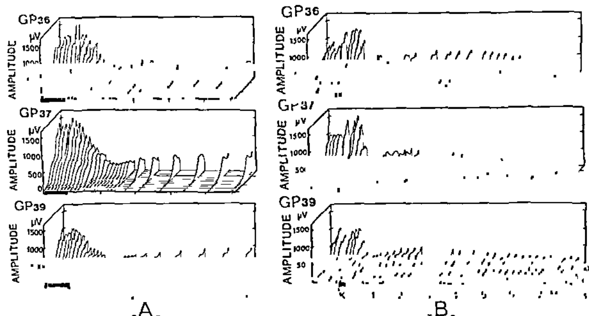


Fig 2 Short term (A) and long-term (B) evolution of the input–output amplitude function in the 3 treated guinea pigs (click stimulation)
C ⚬ Threshold of the response, ■, duration of the Kanamycin treatment) GP36 shows occasional variations due to chronic otitis of the external acoustic meatus but without involving the middle ear, as shown at the time of sacrifice

(1) *The rapid phase* During this short period the responses to the click changed as follows (Fig 2A and 4)

(i) The threshold increased by about 20 to 40 dB

(ii) The latency near threshold increased considerably This shows in fact the absence of N1 for low level stimulation while only N2 is present For this reason the latencies of N1 and N2 have been represented separately on the y input–output functions, the thick line joining the points which correspond to the latency of the more negative (or larger) peak (large dots) On the normal responses, before treatment, this is the latency of N1 (Fig 1 and others before the treatment), but on these altered responses one can see that the largest response

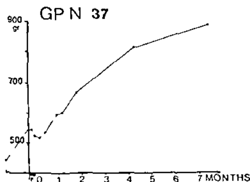


Fig 3 Evolution of the weight of GP 37 during the experiment (■, duration of the Kanamycin treatment)

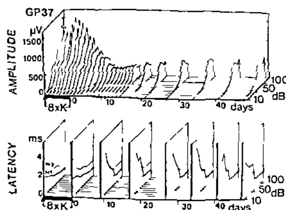


Fig 4 Short term evolution of the input output amplitude and latency functions for GP 37 (click stimulation) The thick line on the latency diagrams joins the latencies of the larger peak in the responses (large dots), which is no longer N1, after the treatment, near threshold, but which is still N1 at high intensities

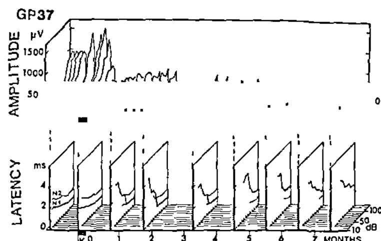


Fig 5 Long term evolution of the input–output amplitude and latency functions for GP 37 (click stimulation) One can observe, on the latency diagrams, the progressive *disappearance of* N1

near threshold and for some decades above, corresponds to N2 until N1 appears and overcomes N2 at higher levels (this transition from N2 to N1 at 70 dB is shown in Fig 4, 9 days after the end of the treatment)

(iii) The overall amplitude (which is measured peak-to-peak without respect to N1 or N2) decreased very markedly both at low and at high intensities The plateau in the amplitude *ut–output function disappears and the curve* ed an exponential form

v) From the threshold of the response to e threshold of N1, the response was broad and slow (Fig 6)

All these features correspond to the *dissociated* pattern observed in man in cases of high frequency hearing loss During the following months the responses remained *dissociated* but with various degrees of dissociation depending upon the stage of the evolution

(2) *Recovery* this abrupt decline of the responses stopped very suddenly and from the 10th day after the treatment the threshold (and in particular the maximum amplitude) improved steadily for about 2 to 3 months However, the responses always presented the *dissociated* characteristics (Figs 3, 2B, 5)

(i) The responses to the 8 000 and 4 000 Hz filtered-clicks recorded after 3 months were clearly *recruiting* (elevated threshold, short latency at threshold, fast increase of the amplitude) suggesting that only N1 was observed with these high frequency clicks (Figs 6 and 7)

(ii) The responses to the 2 000 and 1 000 Hz filtered-clicks were far less altered the amplitude was slightly diminished but the latency was greater (Fig 7)

(3) *The slow phase* this recovery process slowed down, levelled off, stopped and then the responses to the click started again to deteriorate *very slowly but progressively*

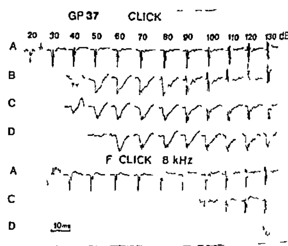


Fig 6 Comparison of the responses to the click and to the high frequency filtered click (8 000 Hz), in GP 37 before the treatment (A), at the end of the rapid phase before recovery, about 10 days after the end of the treatment (B) 3 months (C) and 7 months (D) after the treatment (Negativity downward amplification adjusted so that the traces have somewhat similar amplitudes, for the amplitude in µV, see Fig 7)

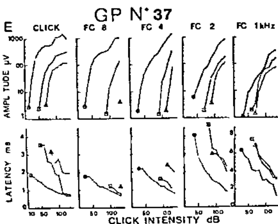


Fig 7 Input–output functions of amplitude (logarithmic scale) and latency of the largest negative peak (linear scale) of the responses to the click and the various filtered clicks (FC) for GP 37 O, before □, 3 months and △, 7 months after the treatment (Note the different scale for the latency for the 1 kHz F C.)

(i) The threshold was elevated to 70 dB

(ii) The latency near threshold was always long

(iii) N1 diminished considerably after $4\frac{1}{2}$ months it was smaller than N2 and disappeared completely after 7 months (Fig 5)

(iv) The response was slow and broad at any click level (Fig 6) and the amplitude very small about 100 μV

(v) The responses to the 8 000 Hz filtered click disappeared almost completely (Fig 7), the responses to the 4 000 Hz were profoundly reduced while the responses to the 2 000 and

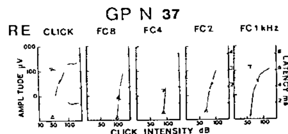


Fig 8 Input–output functions of amplitude (△) (logarithmic scale on the left) and latency (△) of the largest negative peak (linear scale on the right) of the control responses to the click and the various filtered-clicks recorded from the right ear of GP 37 (round window) before sacrifice, 7 months after the treatment. (Note the different scale for the latency for the 1 kHz F C)

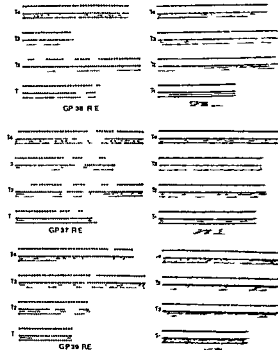


Fig 9 Cochlear cytograms of [illegible] the 3 guinea pigs (●, miss [illegible] turns of the cochleas (T1 4)

1 000 Hz filtered-clicks [illegible] as at 3 months after [illegible]

Control recordings [illegible]

The responses to the [illegible] recorded on the [illegible] were identical to [illegible] but generally better [illegible] plitude) and sma[illegible] observed with the [illegible] (Fig 8)

Cochlear cytogr[illegible]

The phase co[illegible] a the cochleas ([illegible] al , 1964) sh[illegible] ne of hair cell [illegible] ne Corti the [illegible] 10 most comp[illegible] her internal ha[illegible] ase turns were [illegible] ame were almo[illegible] die [illegible] men [illegible] Ant

row of outer hair cells was destroyed For the other turns there was only a sparse destruction of outer hair cells (Fig 9)

DISCUSSION

Response patterns

From the end of the treatment the responses to the clicks were *dissociated* and remained so during the whole experiment to a greater or lesser degree

The responses at the end of the experiment were in complete agreement with the pattern of hair cell damage in the organ of Corti the first turn was extensively destroyed and, at most, only very small responses to the 8 000 Hz filtered-click could be recorded On the other hand the upper turns were preserved and the responses to the low frequency filtered clicks were less modified (only the elements coming from the first turn were actually absent in these responses to the low frequency filtered clicks these elements should have a shorter latency since the first turn is stimulated before the others, this is the eason for the longer latency of the responses after truction of the first turn) The slow and late nses to the unfiltered click are thus made only the responses of these upper turns which are stimulated by the low frequency components of the click The lack of response from the base, obvious with the 8 000 Hz filtered click, appears also with the unfiltered click where there is no short latency peak (N1) at any sound level

This also demonstrates that the click evoked compound VIII nerve responses are elaborated by the entire cochlea in spite of the location of the electrone (first turn) and the transient nature of the stimulus

The responses as they appear 3 months after the end of the treatment are also very instructive In effect at this time the responses to the click were clearly dissociated around 80 dB with long latencies below this level (N2) and short (normal) latencies above (N1) Moreover with the 8 000 Hz filtered click only N1 is observed (short latency) with an elevated threshold (80–90 dB) and the responses are *recruiting* Numerous histological studies have shown that in general only the outer hair cells of the base were affected after such treatment and when the animal is killed at a similar time (Darrouzet, 1967, Hawkins, 1959, Hawkins et al, 1967, Kohonen, 1965) In particular, comparison with the results of Gonzalez et al (1972) is very appropriate since they observed the organs of Corti in guinea pigs immediately, and 70 and 140 days after Kanamycin treatment Although the daily doses were different (250 mg/kg instead of 400) the overall amount of drug is very similar since they prolonged the treatment over 12 days (instead of 8), and the results at 140 days are very similar to ours at 7 months outer and inner hair cells missing at the base This similarity is very likely true also for 70 days and 3 months At 70 days only the outer hair cells of the base were destroyed, so that the *recruiting* response observed to the 8 000 Hz filtered click after 3 months must be coming from the remaining inner hair cells of the base The responses to the unfiltered click after 3 months are made up of the responses of the nerve fibres activated by the outer hair cells of the upper turns of the cochlea (below 80 dB) and of the responses induced by the inner structures of the whole cochlea, mainly the first turn, above 80 dB (short latencies) (N1)

These results at the level of the compound VIII nerve response are also in agreement with the results obtained by Kiang et al (1970) in recording single units activity in VIII nerves of cats treated by Kanamycin where no units were found to have characteristic frequencies that correspond to regions where hair cells were missing , while ' units with normal response characteristics have characteristic frequencies that correspond to regions where hair cells are normal '

Indeed these results cannot be directly related to the subsequent hearing impairment However similar *dissociated* responses have been observed in man with evident high frequency hearing loss On the other hand Stebbins et al (1969), were able to correlate very precisely pure tone thresholds obtained by operant con

ditioning in monkeys with the pattern of hair ell damage in the cochlea after Kanamycin and eomycin treatment These various observations are all in complete agreement

Mechanisms of Kanamycin ototoxicity

These chronic observations of VIII nerve reponses appear very convenient for the study of he evolution of cochlear impairment after Kanaycin treatment, and have revealed various teps which can be interpreted as follows

acute intoxication due to the direct action of he Kanamycin when its level is high after the massive but short administration of the antiiotic

metabolic impairment of the organ of Corti ffecting the survival of the hair cells during he 2 months of potential recovery it can be upposed that Kanamycin has been cleared from he cochlea however the slow but inexorable lteration of the responses which follows the ncomplete recovery resembles an accelerated igeing process

In former chronic electrophysiological studies uch a recovery process has rarely been observed only slight occasional recovery of CM mentioned by Ouchi & Ohtani (1969), and the following long term evolution had not yet been de cribed The long term behavioural studies of tebbins et al (1969), have not shown this type of development, but apparently this is due to he low dose and long period (up to 180 days) f drug administration which must have imaired any recovery process This process, and he following phase of slow deterioration, have o be examined more carefully chronic electrohysiological recordings can monitor these phaes but only biochemical studies performed at he right times can give clues on the etiopathoenic process

Finally, two side remarks must be made also oncerning the results of this study (1) the imilarity of the results for the 3 guinea pigs is triking when other studies have emphasized the ndividual susceptibility to antibiotic damage his could be attributable to the fact that the uinea pigs in this study were screened through the previous surgical procedure, which is not always successful owing to per- and post surgical complications (2) The responses recorded from the right ears just before sacrifice were better than those of the left ears This is certainly due to the fact that the left ears were continu ously stimulated during the entire experiment (and up to 130 dB pe SPL) while the right ears were not This slight over stimulation during antibiotic treatment must have added to the antibiotic toxicity, as it is well known that the cochlea, during such treatment, is more fragile with respect to acoustic trauma (Darrouzet & de Lima, 1962, Dayal et al, 1971)

RESUME

Le potential d action composite du nerf auditif en réponse a des clics et des clics filtres a ete regulierement enregistre durant 7 mois apres traitement de Kanamycine (8 jours 400 mg/kg) chez 3 cobayes équipés d une elec trode à demeure sur la fenetre ronde (oreille gauche)

Des la fin du traitement les réponses au clic devien nent *dissociees* a des degres divers en trois phases principales 1) une phase rapide (8 à 10 jours) pendant laquelle les reponses etaient dramatiquement alterees, 2) une phase de recuperation qui durait pres de deux mois 3) une phase lente pendant les 5 mois suivants durant laquelle les reponses diminuaient lentement mais inexorablement tandis que la composante N1 dans la reponse de meme que les reponses au clic filtre 8 000 Hz (qui etaient devenues *recrutantes* des la fin du traitement) disparaissaient progressivement Les reponses aux clics filtres basse frequences sont moins modifices Les obser vations au microscope a contraste de phase ont montre une destruction quasi totale des cellules ciliees externes et internes du premier tour

ZUSAMMENFASSUNG

Das zusammengesetzte Aktionspotential des Gehornervs bei der Beantwortung von Knackgerauschen und filtrier ten Knackgeräuschen ist wahrend einer Zeitspanne von sieben Monaten nach Kanamycine Behandlung (8 Tage 400 mg/kg) regelmassig bei drei Versuchstieren registriert worden denen eine Elektrode in die Fenestra cochlea (linkes Ohr) eingepflanzt worden war

Nach Beendigung der Behandlung werden liessen sich die Reaktionen auf die Knackgerausche in drei verschiedene Hauptphasen einleiten 1) Eine schnelle Phase (8–10 Tage) wahrend der die Reaktionen in dramatischer Weise sich änderten 2) eine Ruckkoppelungsphase welche nahezu zwei Monate anhielt 3) eine langsame Phase in den folgenden funf Monate wahrend der die Antwortreaktionen langsam aber unerbittlich abnahmen, jedoch der N_1 Bestandteil in der Antwort sowie die Ant

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PERSTIMULATORY SUPRATHRESHOLD AUDITORY ADAPTATION IN CHILDREN

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(Rece ved February 2 1974)

Abst act The decl ne n loudness of a pure tone was used as a cr terion of *perst mulatory suprathresho'd* d tory adaptat on The test ng techn que cons sted of ont nuous b naural balanc ng of a steady adapt ng st mulus w th an interrupted compar son tone of equal frequency the pulse and pulse ntervals be ng of 200 msec durat on The measurements were carried out in the major ty of cases at 60 dB ensat on l vel for fre quenc es 2 000 to 4 000 Hz. In ormal hear ng test ects *younger than 15 years s compared w th* lts adaptat on was of a d fferen haracter It could be demonstrated only in few cases w ch were almost hout except on among the oldest in the r group The nt of adaptat on n the group of 15–18 year old ubjects d d not d ffer stat st cally from that of the adult ons even though the adaptat on was sl ght n 25 . of the cases The lack ng or sl ght adaptat on was not ce also n all types of hear ng d sorders n the young at ents

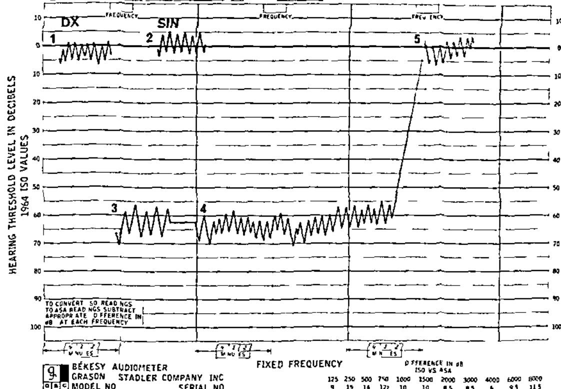
stimulatory suprathreshold auditory adapta is a very stable phenomenon in normally adult persons (Hood 1950 Jerger 1957 t 1958) even though ind vidual d fferences in the amount of adaptation can be large The reatest adaptation values were measured (Karja 968) at 60 and 80 dB SL (sensat on level) for requencies 2 000 to 6 000 Hz the averaged oudness level loss be ng statistically the same 26 9 29 2 dB) In 5 % of 38 test subjects aged 5 years w th an average of 25 4 years adap on was slight less than 10 dB in all test uations Measurements were carried out also young test subjects and the first time a fference in the amount of perst mulatory suprathreshold adaptation between the children and adult test subjects was reported Slight or lacking adaptation was later demonstrated also in conductively deafened young patients (Karja 1970)

n s work was a ded by a grant from the Nat onal h Counc l for Med cal Sc ence

MATERIAL AND METHODS

The decrease in loudness level of a continuous pure tone stimulus was used as a criterion of suprathreshold adaptation The testing technique was based on b naural balancing of the adapting tone throughout the stimulation with an inter rupted comparison tone of equal frequency the pulse and pulse intervals being of 200 msec duration Measurements were carried out with a mod fied Grason Stadler Model E 800 audiometer

At first the test subject determined his thresh old values for each ear at a test frequency with an interrupted tone This was followed by pre stimulatory balancing an interrupted stimulus at the desired sensation level was presented to the ear under test and at the same time a com parison tone was fed into the other ear the pul ses being simultaneous The test subject was asked to adjust the intensity of the comparison tone until its loudness equalled that of the stimu lus. An *adapting i e a continuous stimulus* was then introduced on the experimental ear for 3 min Balancing with an interrupted tone was continued throughout the stimulation along the same principle as in the prestimulatory balance The test subject recorded the recovery of thresh



1 Perstimulatory suprathreshold adaptation measured at 60 dB SL 4 000 Hz on the left ear of a normal-hearing boy aged 12 years Above on the left are shown the threshold tracings for each ear (1, 2) and below the prestimulatory balance (3) In the middle the adaptation tracing (4) shows adaptation only 3 dB during 3 min The recovery of the poststimulatory threshold for the left ear appears at the extreme right (5)

old of the experimental ear for 60 sec with an interrupted tone Fig 1 shows the result of a typical test run for a young normal hearing test subject

Before adaptation measurements air and bone conduction thresholds were determined by the usual descending–ascending method using Madsen Model OB 60 audiometer calibrated to ISO 1964 readings Speech reception thresholds and speech discrimination were measured as described by Palva (1952) Adaptation at threshold level was studied with self-recording Grason–Stadler audiometer by letting the test subject determine his continuous tone threshold for 3 minutes at the same frequencies as the suprathreshold adaptation was measured

Loudness recruitment—in the cases with sensorineural deafness—was studied by the Fowler or Reger method, or in cases with insufficient threshold differences, taking the amplitude size of the threshold tracings as criterion by a self-recording audiometer (Palva, 1957)

The normally hearing material consisted of two groups of young test subjects The age in the first group of 42 subjects varied between 7 and 14 years and averaged 11 8 years, and in the other of 25 subjects from 15 to 18 years with an average of 16 5 years In addition, 19 patients younger than 15 years with conductive deafness and 29 with various types of sensorineural hearing loss were studied

Table I *Perstimulatory suprathreshold adaptation (dB) at 60 dB SL for frequencies 2 000 and 4 000 Hz in normal hearing subjects aged 7–14 and 15–18 years*

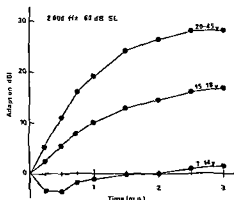
Adapt time (sec)	7–14 years (averaged 11.8 y)				15–18 years (averaged 16.5 y)			
	2 000		4 000		2 000		4 000	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
15	−3.6	3.5	2.7	4.5	2.3	4.9	1.9	3.4
30	−3.5	4.2	−3.2	5.2	4.6	7.0	5.2	8.5
45	−2.6	4.8	−2.5	6.1	7.2	9.1	11.0	13.6
60	−1.7	5.2	1.3	6.6	9.9	10.5	13.4	15.4
90	0.2	5.0	−1.3	7.1	11.6	10.5	16.0	15.9
120	0.2	6.6	0.5	8.0	14.1	11.4	18.3	16.4
150	0.9	7.1	1.6	8.5	15.4	12.4	19.2	17.1
180	1.8	8.4	1.7	9.8	16.1	12.4	20.4	17.0

RESULTS

Normally hearing subjects

All subjects had threshold values better than 20 dB for frequencies 125 to 8 000 Hz. The measuring procedure requires sustained concentration and it appeared that the interest and patience of the youngest subjects sufficed for only one or two recordings. For this reason the tests were limited mainly to 2 000 and 4 000 Hz at 60 dB SL on the experience gained in the adults as regards ease of comparison and amount of adaptation. In the group of 7–14-years old subjects the values at 2 000 Hz are means for 25 cases and at 4 000 Hz for 36. In the age group of 15–18 years, 25 ears were tested at both frequencies.

It appeared that practically no adaptation was demonstrable in subjects younger than 15 years (Table I). Control studies were carried out also at 20 and 40 dB SL and no adaptation was found at these sensation levels either. Fig. 2 shows the results compared with those measured on subjects from 20 to 45 years with an average of 25.4 years (Kärjä, 1968). The initial negative values for the group of the youngest subjects are artificial, they are accounted for by the automatic attenuation mechanism of the audiometer: the absence of response of the test subjects for 1–2 seconds at the beginning of stimulation means decline of the tracing to negative values of that amount. Adaptation in excess of 10 dB was found only in 5 cases the highest value being 28 dB. These test subjects were almost without



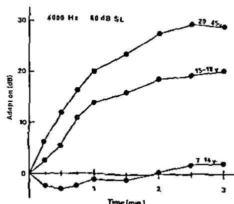


Fig. 2. Perstimulatory suprathreshold adaptation at 60 dB SL for frequencies 2 000 and 4 000 Hz in two groups of young test subjects compared with the results measured on 20–45 year-old persons.

Table II *Prestimulatory balance levels (dB) at 60 dB SL for 2 000 and 4 000 Hz, and corresponding poststimulatory threshold shifts 1 min after cessation of adapting stimulus*

	7 14 years (averaged 11 8 y)				15–18 years (averaged 16 5 y)			
	2 000		4 000		2 000		4 000	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Prestimulatory balance	61 6	11 8	63 5	9 7	59 0	8 2	62 4	8 0
Poststimulatory thr shift	4 6	4 2	4 6	5 4	3 6	3 6	3 3	3 2

exceptions among the oldest in their group In the older age group on the contrary, only 5 cases (20 %) adaptation was less than 10 dB the corresponding percentage among 20–45 year old subjects being 5 % The difference in the amount of adaptation between the youngest and the other two groups was statistically significant ($P<0\ 01$) for both frequencies but not between the two oldest age groups

The prestimulatory balance levels and post stimulatory threshold shifts showed no difference in the statistical tests neither were there any differences as compared to the group of adult test subjects (Karja, 1968)

Table III presents the excursion amplitudes e prestimulatory and poststimulatory thresh tracings as well as those of prestimulatory lance and adaptation curves The only statis tical differences ($P<0\ 05$) between the two groups were in prestimulatory threshold ampli tudes which were wider for the younger test subjects The same fact held true also for the adult persons (Karja 1968) In every other respect the results were equivalent between the three groups of the test subjects The adaptation at threshold level for 22 subjects younger than 15 years tested at 4 000 Hz (6 7 dB) and for 17 at 2 000 Hz (4 3 dB) were of the same mag nitude as in the age group of 15–18 year old subjects (3 9 and 4 5 dB respectively)

Hard of hearing patients

Table IV lists the patients younger than 15 years with various types of hearing disorders The conductively deafened patients had bone con duction values better than 20 dB at 250 to 4 000 Hz Among 19 patients 17 suffered from chronic ear disease and two from otosclerotic (verified later operatively) 19 ears from 29 with sensori neural deafness showed loudness recruitment Adaptation at threshold level was less than 30 dB in all cases except in one referred to later on Speech perception was in most cases better than 80 % only in four ears lower (47 % 67 %) In conductive deafness suprathreshold adaptation measurements were carried out at 60 dB SL Two patients (Table III) with chronic ear disease experienced a loudness level loss more than 10 dB A boy aged 14 years showed greatest adap- tation amounting to 34 dB

Table III *Amplitudes (dB) of pre- and poststimulatory thresholds and excursion widths of prestimu latory balance and adaptation tracings at 60 dB SL for 2 000 and 4 000 Hz*

	7 14 years (averaged 11 8 y)				15–18 years (averaged 16 5 y)			
	2 000		4 000		2 000		4 000	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Prestimulatory threshold	7 2	2 4	6 9	2 1	5 8	1 5	5 7	1 2
Prestimulatory balance	7 4	2 0	7 9	2 7	6 9	2 2	7 0	1 6
Adaptation tracing	7 8	2 7	7 4	3 0	8 1	3 2	8 3	2 8
Poststimulatory threshold	7 1	1 8	6 0	1 6	5 7	1 6	5 5	2 0

Table IV *Perstimulatory suprathreshold adaptation in hard of-hearing patients younger than 15 years*

Diagnosis	Number of cases: Total	Patients with adaptation more than 10 dB
Conductive deafness	19	2
Sensorineural deafness	29	6
Cochlear lesion		
Noise injury	9	1
Infectious lesion	4	
Infratentorial tumour	2	1
Mixed group		
Congenital	11	4
Skull injury	1	
Non-defined	2	
Total	48	8

The lacking or slight adaptation was noticeable also in all types of sensorineural hearing loss of the young patients In 6 cases from 29 studied adaptation exceeded 10 dB In 16 years tests were carried out at 60 dB SL and in 13 at 40 dB SL The greatest adaptation, 45 dB at 2 000 Hz 60 dB SL was found on a 12-year-old boy with incomplete loudness recruitment in an ear with a congenital hearing loss, the threshold loss at 2 000 Hz being 35 dB and the adaptation at threshold level 7 dB In an 11-year-old boy with pontine ependymona adaptation at threshold level amounted to 67 dB However, loudness level loss was no more than 15 dB at 2 000 Hz 60 dB SL His threshold hearing loss was 25 dB, speech discrimination 67 % (in the other ear 93 %), in filtered speech test mon aurally 35 % and binaurally 44 % (in the other ear monaurally 57 %)

DISCUSSION

The difference in perstimulatory suprathreshold adaptation between the young and adult test subjects is surprising It is not due to a possible unreliability of results obtained with a self-recording audiometer in children The results were consistent with those of adults in every respect except that the young subjects had larger amplitudes of threshold tracings That may be explained by the greater experience of the adult test subjects with the recording technique resulting from more numerous test occasions

Perstimulatory suprathreshold adaptation has another subjective characteristic besides loudness level decline, namely a change in quality of pure tone stimulus (de Mare, 1939, Kärja, 1968) It appears to sound lower than the comparison tone, or the stimulus begins to resemble noise Any one of the young test subjects, who showed *no adaptation, was not able to report a subjective* loudness decline or any change in stimulus purity

The poststimulatory threshold curve also proved identical in type with the curve for adults, and there were no statistical differences in the amount of poststimulatory threshold shift 1 min after cessation of stimulation To exclude the possibility of error caused by possible adaptation in the control ear for the comparison tone control tests also were carried out by measuring the loudness level decline only at the end of the third stimulation minute No adaptation was found by this means either

Auditory adaptation may be modified by central factors and the development of a subjective absolute intensity constant as referred to by Egan (1955) but these factors are not of such magnitude as to explain the results

According to Leibbrandt's studies (1964 and 1965) on guinea pigs, auditory adaptation is based on the activity of the efferent acoustic bundles, Hyvarinen (1966) and Hyvärinen et al (1967) considered after electrofysiological measurements maturation of the rabbit's inhibitory interrelationships of the brain cells to occur at puberty Karhunen (1973) observed in histological studies axonal remodelling to occur into the adult age in Deiter's nucleus of the rat This could be the fact also in auditory system The efferent bundles constitute the periferal portion of the inhibitory system of the auditory pathways Thus development of full activity of the efferent connections at puberty could be one explanation of the observed results Another

possible explanation is that cellular metabolism may be significantly different during the growth period as compared with adult life hormonal and enzymatic factors might play a part

Theoretically perstimulatory suprathreshold adaptation in conductive deafness is of equal degree as in normally hearing subjects—it is for the most part a cochlear phenomenon As one could expect, adaptation on young patients with conductive deafness remained the same as measured on the normally hearing subjects

In cochlear lesions of adult patients adaptation was slighter than in normally hearing ears, pronounced adaptation values were found only in retrocochlear deafness (Karja, 1974) Thus on children abnormal values could be expected at least in retrocochlear deafness This material included one case with pontine ependymoma showing very large threshold tone decay, 67 dB at 2 000 Hz The suprathreshold adaptation was a little higher than in the majority of perceptively deafened children, 15 dB at 2 000 Hz 60 dB SL

Adaptation at threshold level was of the same magnitude in both age groups as in normal-hearing adults measured with automatic audio- ter (Palva & Palva, 1961) Very likely that nomenon can be to the most part on ex ession of katabolic metabolism of the auditory system to acoustic stimulation Averaged perstimulatory suprathreshold adaptation on the youngest subjects was of the same magnitude as the threshold tone decay during the equivalent time period

Perstimulatory suprathreshold adaptation might represent a regulation mechanism, mainly by means of the efferent bundles, of the response to a continuous acoustic stimulus, and so it might be partly the result of a development connected with maturation Its meaning according to Ranke s adaptation theory (Ranke, 1955, Keidel, 1961) could be to produce a purposeful balance between stimulus and response to stimulation so that sensitiveness of the receptor organ would remain maximal during continuous stimulation

ZUSAMMENFASSUNG

[illegible]

aus 200 msec langen Impulsen und ebenso langen Intervallen bestand. Die Messungen wurden hauptsächlich bei 60 dB uber den Luftkonduktionschwellen fur die Frequenzen von 2 000–4 000 Hz gemacht

Bei normalhorenden Versuchspersonen die weniger als 15 Jahre alt waren war die Adaptation sehr verschieden von der bei alteren Personen Bei ihnen konnte man sie nur dann zeigen wenn sie ausnahmsweise eintrat In der Gruppe von 15–18jahrigen Personen war die Adaptation sehr gering noch bei 25 % von den Gepruften aber die durchschnittlichen Werte lagen statistisch nicht mehr unter denen der Gruppe der 20–45-jährigen Versuchspersonen Die geringe oder ausbleibende Adaptation war also kennzeichnend fur alle Typen von Schwerhorigkeit der jungen Patienten sowohl bei Schalleitungstorungen als auch bei perzeptiver Schwerhorigkeit

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EINE TESTANORDNUNG FÜR DAS RÄUMLICHE HÖREN

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Abstrakt. Es wird eine Anlage vorgestellt, die dem räumlichen Charakter des Hörens gerecht wird. Die Versuchsperson, deren Augen verdeckt sind, zeigt auf die vermutete Lokalisation der Schallquelle (WN), die nahe ihrem Kopf rechts bzw. links gehalten wird. Völlig geräuschlos werden für alle drei Anteile des subjektiven polaren Koordinatensystems (horizontal, vertikal und Tiefe = Radius) getrennt die durchschnittliche Mittenverschiebung (Verlagerung) und die Streuung der Einzelwerte, welche den Fehler repräsentiert, bestimmt. Es zeigt sich, daß bei 45° seitlicher Untersuchung der Fehler in der Horizontalen nur geringfügig kleiner ist als der in der Vertikalen. In der Tiefe des Raumes sind die Leistungen der 21 Versuchspersonen stark unterschiedlich, während einige Probanden in dieser Dimension e überraschend gute Orientierung zeigten, hatten an den Eindruck, das Geräusche komme von weit halb der Camera silens. Diese Untersuchungsanord g soll zur Erforschung des subjektiven akustischen aumes beitragen.

Unter den Bezeichnungen „Richtungshören" und „räumliches Hören" werden üblicherweise Versuche verstanden, die Teilgebiete dieses komplexen Geschehens erfassen. Von vielen Autoren wurde die Lateralisation untersucht, die einerseits eine starke Vereinfachung des natürlichen Vorganges darstellt, andererseits den Vorteil der Anbietung der Stimuli über Kopfhörer bietet (Keidel, 1966). Schwieriger durchzuführen sind die Experimente im freien Schallfeld, welche jedoch den natürlichen Gegebenheiten näher kommen. Die überwiegende Mehrzahl der Autoren experimentiert in einer Ebene, wobei zumeist die Horizontalebene gewählt wird, eine ausführliche Darstellung der diesbezüglichen Literatur findet sich bei Preibisch-Effenberger (1967), etliche Autoren studierten isoliert die Vertikalebene (z. B. Angell & Fite, 1901). Die Tiefe des subjektiven Raumes des Menschen (polares Koordinatensystem) war bisher nur selten Gegenstand von Untersuchungen (Hornbostel, 1926), vielfach wurde sie simuliert durch Veränderungen der Lautstärke und/oder des Frequenzspektrums (Bekésy, 1930). Weiters wurde von einigen Autoren in zwei der drei Anteile des Koordinatensystems gleichzeitig experimentiert (Klingon & Bontecou, 1966, Harris, 1972) — bei der Auswertung der Ergebnisse wurden die beiden Dimensionen jedoch nicht getrennt berücksichtigt. Untersuchungen, die alle 3 Anteile des polaren Koordinatensystems des subjektiven Raumes des Menschen berücksichtigen (von Ebenen kann man bei einem polaren Koordinatensystem nicht sprechen), liegen nicht vor, wenngleich gerade diese Untersuchungen mit den natürlichen Gegebenheiten am ehesten vergleichbar wären. Nur bei gleichzeitiger Berücksichtigung des horizontalen sowie des vertikalen Winkels und der Tiefe des Raumes, also des Radius, kann man von einer Testung des räumlichen Hörens sprechen. Dieser Ausdruck scheint in der Literatur zwar bereits mehrfach auf, jedoch für Versuchsanordnungen, die nicht der Dreidimensionalität entsprechen. Die vorliegende Studie demonstriert eine dafür geeignete Untersuchungseinrichtung mit den ersten, an Normalhörenden gewonnen Ergebnissen.

METHODIK

Für das Richtungshören wurden bisher von den verschiedenen Autoren drei prinzipiell mögliche

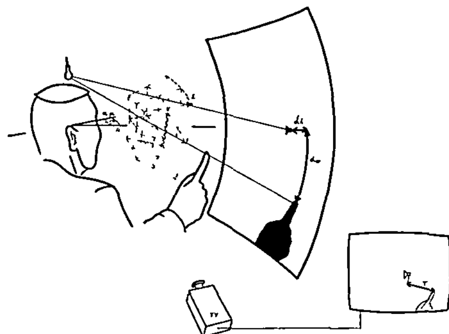


Abb 1 Die Abbildung stellt die Untersuchung der rechten Seite dar Die durch Lichtstrahlen markierten 27 Raumpunkte sind zu sehen der Lautsprecher (*L*) wird einen dieser Punkte gehalten Die Fehlanzeige des Probanden wird in 3 Dimensionen zerlegt der horizontale und der vertikale Winkel (*αh* und *αv*) werden auf dem netzartigen Schirm abgelesen der Fehler in der Tiefe (*T*) ist auf dem Fernsehschirm ersichtlich

Versuchssituationen angewandt (Lateralisations -experimente mit Kopfhorern ausgenommen)

1) die Bestimmung des kleinsten erkennbaren Winkels (Grenzwinkels) in der anglo amerikanischen Literatur minimum audible angle (m a a Sivia & White 1933 auch Preibisch Effenberger 1967)

2) das Hinzeigen auf die vermutete Lokalisa ion ohne optische Kontrolle also mit verdeck i Augen (Walsh 1957) oder mit verdeckter Anlage (*Tonning 1970*) *die Unsichtbarmachung* der Anlage leitet uber zu Punkt

3) d e optische Hilfe die Versuchsperson sieht Anzahl Lautsprecher und kann den als challquelle vermuteten Lautsprecher mit einer Nummer oder sonstwie benennen (Jongkees & er 1958 Fritze et al 1973)

Um einerseits der Dreidimensionalitat zu ge und andererseits keine besonderen Anfor ungen an den Probanden zu stellen schien von den verschiedenen Untersuchungsmoglich eiten das *blinde Hinzeigen auf die vermutete* ation des Lautsprechers das gunstigste erfahren zu sein

Die verwendete Anlage ist in einer mit Glas fiberkeilen ausgekleideten camera silens untergebracht Der Kopfmittelpunkt der Versuchsperson ist das Zentrum des subjektiven Polarkoordinatensystems (Abb 1 u 2) Der Kopf der Versuchsperson liegt ruckwarts und oben an einer Stutze an — minimale Bewegungen sind also moglich Die Augen der Versuchsperson sind durch kleine undurchsichtige Kappen verdeckt welche die Form des Kopfes praktisch nicht verandern *Die Gerauschanbietung erfolgt durch* einen sehr kleinen Lautsprecher (2 cm Schallaustrittsoffnung 2 mm) der — auf einem kleinen Stabchen montiert — vom Untersucher gehalten wird (Der Untersucher tragt zur Verminderung der Reflexion eine Kleidung aus Wolle) Als Stimulus dient das WN eines Vienatone Audiometers (M 132) mit einem Schalldruck von 50–55 dB das fur die Dauer von 1 sec angeboten wird Die Versuchsperson zeigt unmittelbar danach auf die vermutete Lokalisation der Schallquelle wobei sie angewiesen wird auch die Entfernung zu berucksichtigen

Die Feststellung der Position sowohl des

Lautsprechers als auch des Fingers des Untersuchten erfolgt durch Licht, sodaß der Versuchsablauf mit keinerlei Geräuschen verbunden ist Für die Positionsbestimmung in der Horizontal- und Vertikalebene dient ein netzartiger Schirm aus schallschluckendem Kunststoff und Wolle, der in einer Entfernung von 1 m vom Kopfmittelpunkt des Probanden angebracht ist (Abb 1) und — als Ausschnitt einer Kugel gebaut — dem Polarkoordinatensystem entspricht, er ist 50 mal 50° groß und weist eine Gradeinteilung auf Ein unmittelbar über dem Kopf der Versuchsperson befindliches Lämpchen wirft einen Schatten sowohl des Lautsprechers als auch des Fingers der Versuchsperson auf diesen Schirm Damit ist für die Horizontal- und die Vertikalebene die Lokalisation leicht und rasch zu erkennen Der dabei auftretende Parallaxfehler (Vertikalebene mit Abhängigkeit von der Tiefe) wird bei der Auswertung der Ergebnisse rechnerisch berücksichtigt (Um den Parallaxfehler zu verkleinern ist die bestehende Anlage gegenüber der Abb um 5 cm in der Höhe verschoben, was durch die Korrekturrechnung ausgeglichen wird Aus Gründen der Übersichtlichkeit ist se *Verschiebung in den Abbildungen wegge*-en) Auch in der dritten Dimension, der iefe, wird die Lokalisation durch Licht bestimmt ein Dia Projektor, der sich in einem Winkel von 90° zur Zentralachse der Tonanbietung befindet, projiziert drei Streifen (ebenfalls aus Gründen der Übersichtlichkeit nicht eingezeichnet) für die drei möglichen Tiefenlokalisationen Neben dem Projektor befindet sich eine Fernsehkamera außerhalb der camera silens wird auf dem dazugehörenden Bildschirm die Fehlanzeige in der Tiefe mittels eines Rasters von einer Hilfsperson abgelesen Dabei wird die horizontale Krümmung des polaren Koordinatensystems *nicht berücksichtigt, was allerdings* durch den großen Abstand (2,5 m) der Kamera von der Zentralachse nur einen kleinen Fehler ergibt, der sich herausmittelt Die Positionen der Tonanbietung sind fixiert Horizontalebene die Testung erfolgt rechts und links seitlich in einem Winkel von 45 ± 10° Der Winkel in der Vertikalebene beträgt 0 ± 10°, der Abstand vom Kopfmittelpunkt (Tiefe des Raumes, Radius des Polarkoordinatensystems) beträgt 30 ± 10 cm Insgesamt sind also rechts bzw links 27 mögliche Positionen vorgesehen, von denen dem rechten *und dem linken Ohr je 24 Lokalisationen* (in randomisierter Reihenfolge) angeboten werden, wobei die getestete Seite mehrmals abgewechselt wird Die seitliche Testung (45°) wurde gewählt, da in dieser Stellung die beiden Ohren weitgehend getrennt untersucht werden können (bei frontaler Beschallung und Ausschaltung eines Ohres kommt es zu relativ großen seitlichen

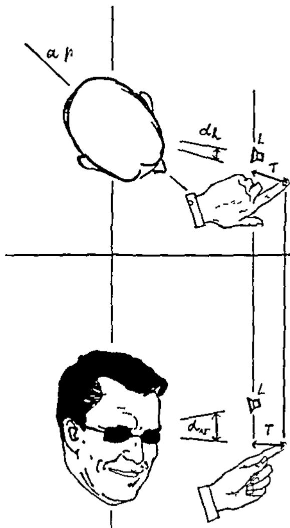


Abb 2 Die Darstellung dieses Vorganges aus zwei Betrachtungsebenen von oben und von der Seite In dieser Darstellung wird die linke Seite getestet es sind nur der Lautsprecher die Hand des Probanden und die drei Dimensionen des Fehlers eingezeichnet

Verschiebungen; bei der Untersuchung in 90° seitlich stört bei der Anzeige die eingeschränkte Beweglichkeit des Armes)

ERGEBNISSE

Die beschriebene Anlage ermöglicht es also, die Fehlweisung in allen 3 Anteilen des dreidimensionalen subjektiven polaren Koordinatensystems getrennt zu bestimmen Inversionen (d h den von der Psychologie her bekannten Spring- ' ' ' vergleichbare Fehl-Lokalisationen vorne/ ' kamen sehr selten vor, jedoch wurde der ion häufig als wesentlich weiter entfernt em- ' nden, und zwar nicht selten außerhalb des Raumes Dieser Eindruck war für die Versuchsperson ähnlich zwingend wie etwa die perspektivischen optischen Täuschungen, die auch durch besseres Wissen in der Empfindung nicht korrigiert werden können (Keidel, 1966) Dies ist eine wierigkeit bei der Bestimmung des Fehlers der Tiefe Es ist jedoch zu beobachten, daß Versuchspersonen den Ton zwar als von außerhalb ihrer Reichweite kommend empfinden, jedoch zwischen näher und weiter unterscheiden können und sich diese Unterscheidung in ihrer Lokalisationsangabe ausdrückt, sie geben gleichsam immer nur einen Teil der Entfernung ı, jedoch einen eher gleichbleibenden Teil In ' esem Fall kann man von einer eingeschränkten Orientierung in der Tiefe des Raumes sprechen ie maximale Fehlweisung für die Tiefe betrug bedingt durch die Länge des Armes) + 60 cm, die sehr seltenen Inversionen wurden als −60 cm gerechnet, um die Streuung nicht durch einen nelnen Wert zu sehr zu ändern Es wurden it fur alle 3 Dimensionen und fur die bei- ı Ohren getrennt die Mittelwerte und die ıanzen bzw die Streuungen berechnet Damit sich pro Versuchsperson 6 Mittelwerte Streuungen und für jeden dieser Mittelwerte liegen 24 Einzelbeobachtungen vor Diese 'erte wurden für 21 Versuchspersonen ermittelt, normalhörend, gesund und rechtshändig , das durchschnittliche Alter betrug 27,3 Die ermittelten Werte einer Versuchsperson sind als Beispiel in Tab I wiedergegeben

Tabelle I

Die Daten eines Probanden (H R , 23a), jedem der 6 Mittelwerte (M) bzw Streuungen (s) liegen 24 Einzelbeobachtungen zu Grunde Ein negatives Vorzeichen bedeutet Verlagerung nach links, bzw nach unten, bzw in geringere Entfernung von der Versuchsperson

M	s	
5,3°	6,7°	rechts horizontal
−3,4°	7,9°	links horizontal
−10,9°	12,6°	rechts vertikal
−4,7°	13,2°	links vertikal
28,0 cm	11,0 cm	rechts Tiefe
19,1 cm	10,5 cm	links Tiefe

Bei der Betrachtung der Ergebnisse muß man berücksichtigen, daß in den drei Dimensionen unterschiedliche physiologische Mechanismen die Lokalisationsfähigkeit ermoglichen (Fritze et al , 1973), während in der Horizontalen vor allem interaurale Intensitäts-, Phasen- und Zeitunterschiede von Bedeutung sind ist die Lokalisation in der Vertikalen und in der Tiefe vorwiegend ein monaurales Geschehen Für die vertikale Dimension ist vor allem die Reflexion des Schalles in der Ohrmuschel von Wichtigkeit, während für die Tiefe die Intensität und die Tonhöhe eine große Rolle spielen

DISKUSSION

Die 3 Anteile des polaren Koordinatensystems müssen einerseits getrennt betrachtet werden, andererseits ist ein Vergleich aufschlußreich Bei diesen Daten entspricht der Mittelwert bis zu einem gewissen Grad der Verschiebung des Mitteneindruckes, die Streuung kann als der Fehler bezeichnet werden Bei der Betrachtung der Daten zeigt sich

Horizontalebene

Bei der Beschallung der rechten Seite wird eher nach rechts, bei der Beschallung der linken Seite mehr nach links fehlgezeigt, (in Tab I bedeutet ein positiver Wert die rechte Seite), es besteht also beiderseits die Tendenz zur Fehllokalisation nach lateral, im Durchschnitt wurde etwas mehr nach rechts (2,1°) angezeigt Dieser

Wert ist zwar schwach signifikant — die Ursache könnte aber in psychologischen Faktoren zu suchen sein, da der Untersucher rechts vom Probanden stand. Einzelne Versuchspersonen hatten aber doch erhebliche Verlagerungen dieses Mittelwertes (Maximal 13,7° bzw. −6,2°) Durchschnittlich betrug der Unterschied zwischen der Testung des rechten und des linken Ohres 11,2°.

Die Streuung (Fehler) betrug horizontal durchschnittlich 7,4°. Dieser Wert mag groß erscheinen — man muß aber bedenken, daß die Untersuchung 45° seitlich erfolgte (Mills, 1958). Die größte Streuung (Durchschnitt beider Seiten) betrug 11,2° die kleinste Streuung 3,5°; in 3 Fällen bestand ein signifikanter Unterschied zwischen rechts und links.

Vertikalebene

Hier wurde eher nach unten fehllokalisiert (negative Werte), im Durchschnitt um −11,0°, die Bedeutung dieses Wertes ist jedoch fraglich. Zwischen der Untersuchung rechts und der links betrug der durchschnittliche Unterschied ,6°, bei 8 der 21 Patienten war der Mittelwert hts von dem links signifikant unterschiedlich durchschnittliche Fehler (Streuung) betrug also nur wenig mehr als der horizontale Fehler (7,4°) — dieser Unterschied ist nur schwach signifikant. es ist also bei der Untersuchung in 35°–55 seitlich die Orientierung vertikal nur *gering schlechter als horizontal. Von Interesse* ist auch, daß in nur 4 Fällen der Fehler rechts von dem links signifikant unterschiedlich war. Der Fehler zeigt also eine bessere Gleichverteilung zwischen rechts und links als der Mittelwert, dieser Tatsache kommt Bedeutung bei der Anwendung dieses Testes für die Diagnostik von Temporallappenläsionen zu (Fritze & Gloning, 1973).

Tiefe des Raumes

(Radius des Polarkoordinatensystems). In dieser Dimension sind die Leistungen der Versuchspersonen stark unterschiedlich. Einerseits gab es überraschend *gute Leistungen*, so erreichte eine Versuchsperson eine durchschnittliche Verlagerung (rechts + links) von nur 0,3 cm (!) bei einem durchschnittlichen Fehler von nur 5,8 cm (Positive Werte bedeuten eine größere Entfernung). Andererseits zeigten 9 der 21 getesteten Personen nur eine geringe Orientierungsfähigkeit, sie empfanden das Geräusch immer als von weit weg kommend und zeigten dementsprechend bei fast allen Anbietungen mit ausgestrecktem Arm an, was durch eine große Verlagerung (z. B. 35,4 cm) bei relativ kleiner Streuung (7,4 cm) zum Ausdruck kommt, wobei jedoch die Normalverteilung nicht mehr gegeben ist. Die durchschnittliche Verlagerung betrug 20,4 cm (positive Werte bedeuten Verlagerung in größere Entfernung), der durchschnittliche Unterschied zwischen rechts und links war 7,5 cm. Die Streuung *betrug im Mittel 9,9 cm. Wesentlich scheint* noch, daß auch hier — noch stärker ausgeprägt als in der Vertikalen — die Verteilung zwischen rechts und links beim Fehler besser der Gleichverteilung entspricht, als bei der Verlagerung, dies hängt wohl mit der zentralen Verarbeitung (Temporalrinde) zusammen.

Vergleich der 3 Dimensionen

Im Durchschnitt aller 21 Versuchspersonen unterscheidet sich — wie erwähnt — der horizon*tale vom vertikalen Fehler nur geringfügig zu*gunsten des horizontalen Fehleranteils (Fritze & Gloning, 1973). Weiters wurden die Streuungen in den einzelnen Dimensionen miteinander korreliert. Ein signifikanter Zusammenhang (r = 0,53) konnte jedoch nur zwischen dem hori*zontalen* und dem *vertikalen Fehler gefunden* werden.

Vergleich der beiden Seiten

Der Vergleich der Streuung der rechten mit der der linken Seite (alle 21 Versuchspersonen zusammen) ergibt für keine der 3 Dimensionen einen signifikanten Unterschied, nicht einmal einen Trend. Man kann also im Durchschnitt nicht von einer besseren Leistung rechts oder links sprechen (alle Versuchspersonen waren rechtshändig). Auch für das Fehlerverhältnis rechts links der einzelnen Versuchspersonen

konnte zwischen den drei Dimensionen kein ignifikanter Zusammenhang gefunden werden

Weitere Untersuchungen sollen den Einfluß erschiedener Faktoren wie Alter und Hörstö-ungen aufzeigen Auch die Anwendbarkeit als iagnostisches Hilfsmittel insbesondere bei Tem-orallappenlesionen erscheint untersuchenswert Fritze & Gloning, 1974)

ur die statistischen Berechnungen wurden der *T*-Test, er *F* Test und die Korrelation angewandt (Cavalli-forza 1969) Mit einer Ausnahme (siehe oben) ent-prach die Verteilung immer ungefähr einer Normal-erteilung Bei $p < 5$ ‰ wurde eine schwache Signifikanz ingenommen Die einzelnen Signifikanzniveaus (1 bzw ‰) wurden aus Gründen der Übersichtlichkeit weg elassen. Für die Berechnungen wurde ein Computer der irma Hewlett Packard (2100 A) verwendet

Außer der für diese Studie verwendeten Bestimmung ler Raumpositionen durch Licht ist die Bestimmung uch durch gleichzeitige Fotographie mit zwei räumlich etrennten Kameras möglich Die Bilder werden aus emessen und elektronisch wird dann von beiden Koor-inatensystemen der Kameras auf das Polarkoordina-ensystem des Probanden umgerechnet Zur Einhaltung timmter Tonanbietungspositionen ist aber auch bei ieser Anlage eine Markierung durch Licht erforderlich

SUMMARY

A test situation which concerns the spatial character of earing is demonstrated The subject indicates the esti iated location of the stimulus The WN is presented t a short distance from the head and thus discrepancies i distance can also be discriminated

The subjective space of man (polar system of coordi ates) consists of three dimensions (horizontal, vertical nd distance – radius) For each of these three dimen ons the average shift of the centre and the standard eviation (representing the error) are calculated

When testing lateral (45° right or left) the average rror is a little smaller for the horizontal scale than for he vertical As far as the distance is concerned the sults of the 21 subjects varied considerably while some f the persons showed surprisingly good location ability, thers supposed the noise to come from even outside ie *camera silens*

The investigation has been carried out to contribute i the exploration of subjective acoustic space It is also lanned to apply the test as a diagnostic procedure in arious neurological disturbances

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SEMI-OBJECTIVE METHOD FOR AUDITORY MASS SCREENING OF NEONATES

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Abstract An instrument called the Accelerometer Recording System (ARS) was developed for graphically recording responses of neonates to auditory stimulation Its main component is an accelerometer, which picks up

offer a semi objective test procedure which is short, simple and reliable It promises to be a valid test method for mass screening of newborns for early detection of profound hearing loss

The importance of early detection of profound ring loss for the successful rehabilitation of hearing-impaired child has been stressed by workers in the field for many years Identification of the deaf in the pre symptomatic stage implies mass screening of all infants In most countries the infant population is available for this purpose only in the maternity ward

Since the introduction of an auditory screening program for newborns by Downs & Steritt in 1967, many hospitals launched similar programs The results of controlled programs were disappointing, because of their high rate of negative errors (Altman & Shenhav, 1971, Ling, 1971) Recently, the Joint Committee of Infant Hearing Screening of the USA has discouraged mass screening programs as routine procedures

The object of this pilot study was the development of an instrument to register graphically the responses of neonates to auditory stimulation The instrument, which was called the "Accelerometer Recording System" (ARS) was designed to improve the efficiency of mass screening programs for the detection of profound hearing loss in newborns

The instrument consists of (1) Sound Source, (2) Cradle, (3) Vibration-Pick up and Analyzing System, (4) Recorder

(1) Sound Source Tape Recorder (Sony TC 200) plays pre-recorded thermal band noise, amplified by Audio-Amplifier (Bell 2122-B) into loudspeaker (Philips 8 inch), which is mounted on an adjustable stand (Fig 1)

(2) Cradle An infant cradle (34 × 60 × 26 cm) was fitted with a "wooden pillow" consisting of a board (26 × 20 cm), supporting head and shoulders of infant The whole cradle is placed on a "floating floor structure" (Fig 2) It consists of a concrete slab, weighing over 50 kg, supported by a tyre tube The concrete slab is covered with a polyurethane sheet The complete structure and cradle were placed on a table (Fig 1)

(3) Vibration Pick-up and Analyzing System An Accelerometer (Bruel & Kjaer 4330) is attached to top of "wooden pillow" A preamplifier (Bruel & Kjaer 2625) amplifies the voltage of the "Response Signal" from accelerometer, which passes through a "Narrow Band Filter" (Krohn–Hite 330 N), adjusted to 0 1–20 Hz

Supported by grant 06-482 2 by the Department of Health Education and Welfare of the USA (Maternal and Child Health Service)

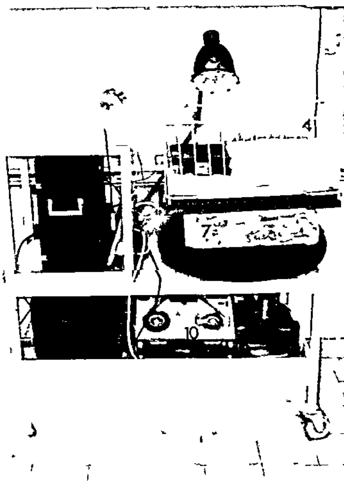
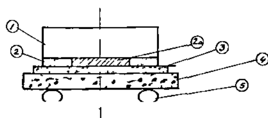


Fig 1 Photograph of Assembled ARS 1 Electrocardiogram 2 Timer 3 Loud speaker on adjustable stand 4 Cradle 5 Wooden headrest with accelerometer 6 Pre-amplifier 7 Concrete slab 8 Rubber tyre 9 Narrow band filter 10 Tape recorder 11 Audio amplifier 12 Polyurethane sheet

(4) Recorder Voltage is registered by the pen-recorder of an Electrocardiograph (Fukuda RS-0-DH) The marker of the recorder notes the stimulus on the paper and is connected th an Automatic Timer", which releases and the duration of stimulus Paper speed recorder was 25 mm/sec

Fig 3 shows a diagram of the ARS



2 Diagram of floating floor structure 1 Cradle Foam rubber mattress 2a Iron disc in mattress 3 lyurethane sheet 4 Concrete slab 5 Tyre tube

TESTING PROCEDURE

Subjects were newborns aged 20–96 hrs, at the Newborn Nursery of the Rambam Hospital, considered healthy by a pediatrician, and with a birthweight between 2 900 and 4 000 g In fants were removed from their cribs and placed into the testing cradle, which was set up in a separate room They remained wrapped in dia pers leaving only head and upper limbs for observation They were classified as (1) awake and quiet , when their eyes were open and they remained quiet after transfer (2) 'deeply asleep ' when not aroused by transfer and (3) in "slight sleep' , when they were temporarily aroused by transfer Fretful or crying infants were excluded

Only neonates aged above 20 hrs were tested, as previous studies had shown a diminished (observed) response rate in younger infants

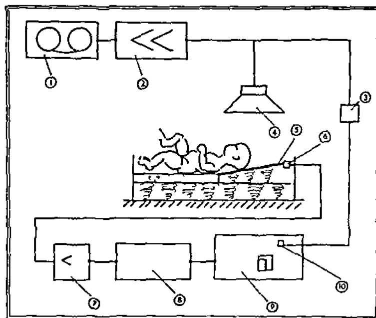


Fig 3 Diagram of ARS 1 Tape Recorder 2 Audio-amplifier 3 Timer 4 Loudspeaker 5 Wooden headrest on foam rubber 6 Accelerometer 7 Pre amplifier 8 Narrow band filter 9 ECG Recorder 10 Marker

Two testers were employed one *observing* the infants' response, the other *operating* the equipment

Sound stimulus was a 1/3 octave noise band with center frequency of 3 150 Hz Sound level was 90 dB at ear of infant Duration of signal , 500 msec Three stimuli or more were pre- with an interval of at least 5 sec

RESULTS

In 400 newborns tested, a distinct recording was obtained in all but seven There was a definite relation between the observed strength of the response and the height of the registered curve

Observation of responses showed whole or partial body movement (as most prominent response) in 235 and eyeblink in 132 Observation failed to detect a response in 33, but it was recorded in all but seven

Graphs 1–6 show typical recordings

In the tested population there were three infants with a known history of deafness in the parents or the other siblings One was examined at the age of 14 days, he suffered also from severe jaundice In all three, observations and recordings showed normal responses One of the seven newborns in whom no recording was obtained, was later found to have three deaf maternal cousins

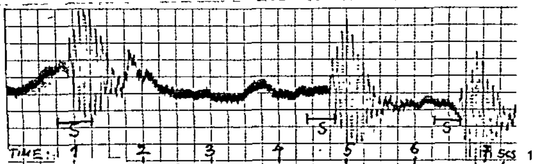
DISCUSSION

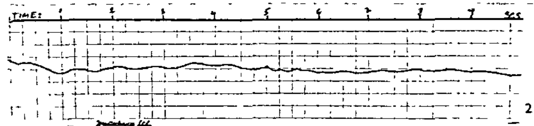
The ARS described is the sixth model developed in a series of experiments It is based on an idea of one of us (L S) and was originally used for the determination of the frequency range of hearing in white rats (Schaudinischky, 1966)

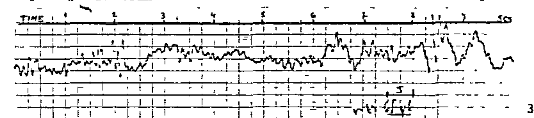
The principle component is the accelerometer used as transducer It generates a voltage proportional to the acceleration of vibrations set u in the wooden pillow The use of acceleratio instead of velocity, considerably increased th instrument sensitivity, with minimal interferenc by undesired side effects Although the instru ment is very sensitive to vibrations, breathin and heartbeats do not show in the recordings as their acceleration is very low

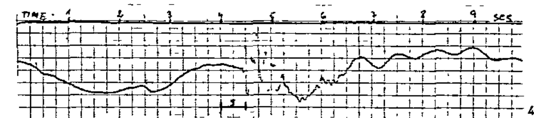
The "Wooden Pillow" enhances pick up o vibrations from weak response movements sucl

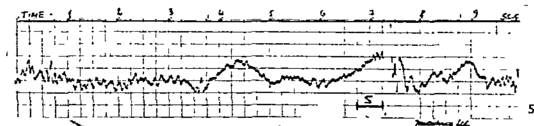
Graph 1 Paperball (1 02 g) dropped on wooden pillow' from 10 cm height *S* Stimulus
Graph 2 Sleeping infant without stimulus
Graph 3 *Fretful infant. S Stimulus Response recor* not distinct from records of ongoing activity
Graph 4 Sleeping infant *S* Stimulus Distinct respons record of whole body movement
Graph 5 Awake and quiet infant *S Stimulus Distinct* record of whole body movement
Graph 6 Sleeping infant *S* Stimulus Distinct record of eyeblink.

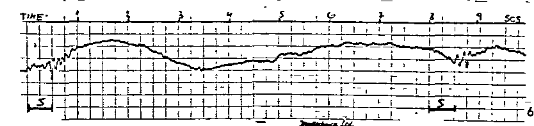

S
S
S
TIME: 1 2 3 4 5 6 7 SCS
1


TIME: 1 2 3 4 5 6 7 8 9 SCS
2


TIME 1 2 3 4 5 6 7 8 9 SCS
S
3


TIME 1 2 3 4 5 6 7 8 9 SCS
S
4


TIME 1 2 3 4 5 6 7 8 9 SCS
S
5


TIME 1 2 3 4 5 6 7 8 9 SCS
S
S
6

as eyeblink It is possible that eyeblink is accompanied by contractions of neck muscles, which elude observation The infants' responses are contractions of striated muscles and the energy of their mechanical reaction is likely to be contained in the range of 0 1–20 Hz They may also be considered as a kind of DIRAC pulse (Papoulis, 1962), which contain a very broad band of frequencies

Adjustment of the filter to the lowest pass band gave a very good signal to noise ratio The influence of speech and other random noises in the testing room and especially that of the sound pulse of the stimulus, was completely eliminated from the recordings Solid borne noise was excluded by placement of cradle on the "floating floor structure"

B Simmons and assoc presented another attempt of semi objective auditory mass screening at the Congress of Audiology in Budapest (October 1972, personal communication) They use a different transducer for registration of response movements It is attached to all cribs in the nursery, testing all infants simultaneously The Cribo Gram' does not require any hand of infants for the test

Tests with the ARS can be carried out by the personnel of the newborn nursery, after a very short training period Each test lasts 2–3 min Records are easily read by physician or nurses

Mass screening of neonates for early detection of deafness, although very desirable, was disclaimed because of the low validity of the test method, which relied on human observation The ARS, as a semi objective method, seems to offer a more reliable testing procedure as shown in this pilot study For full evaluation of the validity of the ARS a large scale mass screening of at least 10 000 newborns with a follow up of 2 years, is needed in order to assess the rate of positive and negative errors

RÉSUMÉ

Un instrument appele Systeme d Enregistrement Accélérometrique a été developpé pour l enregistrement graphique des réponses de nouveau nés a une stimulation auditive Son composant essentiel est un accélérometre qui détecte des vibrations du berceau causées par les mouvements de l enfant en reponse aux stimulations Dans une étude preliminaire chez 400 nouveau nes un enregistrement significatif fut note chez tous à l exeption de 7 Le systeme d enregistrement accelérometrique semble offrir une methode de detection semi objective breve simple et sure Cette technique semble promise a etre une méthode de detection valable pour le depostage precoce de la surdité chez les nouveau nes

ZUSAMMENFASSUNG

Das Accelerometer Recording System (ARS) wurde entwickelt, um Reaktionen von Neugeborenen auf akustische Stimuli graphisch zu registrieren Sein wichtigster Teil ist ein Akzelerometer, das Vibrationen der Wiege erfasst die durch Reaktionsbewegungen des Sauglings hervorgerufen werden

In einer vorlaufigen Untersuchung von 400 Neugeborenen wurde mit dem ARS eine deutliche Reaktion bei allen ausser sieben registriert Das ARS ermoglicht eine semiobjektive Untersuchungsmethode die einfach kurz und verlässlich erscheint Massenuntersuchungen von Neugeborenen zur Fruhdiagnose der Taubheit scheinen mit dem ARS leicht und zuverlässig durchfuhrbar zu sein

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ZUR PROTEINKONZENTRATION DER MEERSCHWEINCHEN-PERILYMPHE

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(Eingegangen am 26 April, 1974)

Abstrakt Die Proteinkonzentration der Meerschweinchen Perilymphe wurde mit Hilfe einer Mikromodifikation der Methode von Lowry et al systematisch untersucht Die Perilymphe der Scala vestibuli und der Scala tympani wurde am lebenden Tier und unmittelbar postmortal nach verschiedenen Methoden gewonnen Am lebenden Tier ist es besonders schwierig, blutfreie Proben zu ge winnen Ein weiteres Problem der Perilymphgewinnung am lebenden Tier ist die Verunreinigung der tympanalen Perilymph Proben mit Liquor Dadurch wird die Proteinkonzentration der Perilymphe artifiziell stark erniedrigt Nach subokzipitaler Eroffnung des Liquor raumes vor der Probenentnahme besteht dagegen zwischen der Proteinkonzentration der tympanalen und der vestibularen Perilymphe kein signifikanter Unter schied mehr Die mittlere Proteinkonzentration betragt in beiden Schneckenskalen ungefahr 150 mg/100 ml Ahnliche Mittelwerte findet man auch bei postmortal ...n Proben intraarteriell perfundierter Tiere ... nicht perfundierten Tieren liegt der tympanale Mittelwert bei postmortaler Entnahme signifikant hoher Die Artefaktursachen bei Perilymphuntersuchungen werden diskutiert

Über die Proteinkonzentration der Meerschweinchen-Perilymphe (PL) liegen in der Literatur bisher zahlreiche Mitteilungen vor (Smith et al, 1954 Lazzaroni 1956, Citron et al, 1956, ...yake, 1960, Rauch, 1964, Schindler et al, ...965, Rademacher, 1966, Schnieder, 1968) Die ...gegebenen Mittelwerte variieren jedoch zwi...chen 42 und 400 mg/100 ml Als Ursache für ...se unterschiedlichen Proteinwerte kommen ...eben unterschiedlichen Bestimmungsmethoden ...ch hauptsächlich Artefakte bei der ...gewinnung in Betracht Wir haben deshalb unsere bisherigen qualitativen Untersuchungen (Scheibe et al, 1972) durch systematische quantitative Untersuchungen erganzt Diese wurden zunächst am normalen unbeschallten Tier unter besonderer Berücksichtigung der Probengewinnung durchgeführt Ziel der Untersuchung war die Klärung folgender Fragen

1 Wie hoch ist die wirkliche Proteinkonzentration der Meerschweinchen PL?
2 Unterscheidet sich die Proteinkonzentration der PL in den beiden Schneckenskalen?
3 Welchen Einfluß hat die Probengewinnung auf das Analysenergebnis?

...ie Untersuchungen wurden im Auftrage des Ministeriums fur Gesundheitswesen der DDR im Rahmen der ... durchgefuhrt.

MATERIAL UND METHODEN

Gewinnung der PL

Die Untersuchungen wurden mit 200–300 g schweren normalhörenden Meerschweinchen (Prufung mit Preyerschem Ohrmuschelreflex) in Äthylurethan-Narkose durchgeführt Die PL wurde unmittelbar postmortal und am leben den Tier nach verschiedenen Methoden gewonnen

Bei der postmortalen Gewinnung wurden die Tiere dekapitiert und die PL durch die übliche Punktion der herausgebrochenen Schneckenkapsel und vorsichtiges Absaugen in der schon beschriebenen Weise (Scheibe et al, 1972) erhalten Bei einigen Versuchsreihen wurden die Tiere zur Gewinnung blutfreier PL vor dem Dekapitieren intraarteriell mit Ringer Lösung

bzw Infukoll® M 40 (VEB Jenapharm Serumwerk Bernburg 100 g Dextran 40+9 g NaCl in 1000 ml) perfundiert

Am lebenden Tier erfolgte die PL-Gewinnung

1 durch die übliche Punktion der Schneckenkapsel und vorsichtiges Absaugen der PL,
2 nach zusätzlichem lokalen Kautern der Punktionsstelle, die dabei auftretenden Temperaturanderungen in der PL wurden von Dahl (1973) durch Messung mittels Mikro-Thermoelementen (Kupfer Konstantan, Spitzendurchmesser ≤0,1 mm) kontrolliert,
3 bei subokzipital eroffnetem Liquorraum, bei diesen Versuchsreihen wurde die tympanale PL durch Punktion des runden Fensters gewonnen

Der Blutgehalt der PL-Proben wurde durch Erythrozytenzählung kontrolliert Danach wurden die Proben zentrifugiert Fur die Berechnung der Proteinkonzentration wurden nur nicht hämolytische Proben mit einer Blutverunreinigung <0,25 ‰ verwendet Die Proteinkonzentration des Serums betrug *4470±665 mg/100* ml ($n=22$) Bei verunreinigten nicht hämolyti- Proben ist also eine artifizielle Protein- hung der PL durch Serumproteine um maxi- al 10–15 mg/100 ml moglich

Serum wurde aus Mischblut gewonnen, Liquor durch subokzipitale Punktion

Proteinbestimmung

Die Proteinbestimmung erfolgte nach Lowry et al (1951) Fur die Messung standen Mikroküvetten von 1 cm Schichtdicke zur Verfugung, die unter den angewendeten Bedingungen ein Fullvolumen von ungefähr 240 μl erforderten Es wurde deshalb ein Meßansatz von 260 μl Gesamtvolumen gewahlt Der Anteil der Proteinlösung betrug dabei 40 μl Unter diesen Bedingungen (λ 750 nm) lagen die Meßwerte bei Verwendung von 1–3 μl PL, die mit physiologischer NaCl-Lösung zu 40 μl ergänzt wurden, im optimalen Extinktionsbereich Bei Liquor wurden 5–10 μl und bei Serum 3 μl (1 50 verdünnt) verwendet Als Leerwert diente der Meßansatz ohne Probe

Zur Probendosierung wurden graduierte Präzisions Mikroliterspritzen (Scientific Glass Engineering Pty Ltd, London) Type 1 B (0 02–1 μl) und 5 B (0,1–5 μl) verwendet

Als Standardprotein diente kristallisiertes Rinderserum–Albumin, reinst, >98 ‰ (Serva, Heidelberg) Für die Eichkurve wurden 10 verschie dene Einwaagen im Bereich zwischen 50–500 mg/100 ml, bezogen auf wasserfreies Rinderserum–Albumin, an verschiedenen Tagen bestimmt Der mittlere Variationskoeffizient betrug im Bereich der Eichkurve VK=8,3±0,9 ‰ ($n=33$) Zur Qualitätskontrolle der Methode wurden verschiedene Eichlosungen laufend mitbestimmt

Von dem Meßwert wurden innerhalb der ein zelnen Versuchsreihen der arithmetische Mittelwert und die Standardabweichung berechnet Die Prufung der Mittelwerte erfolgte mittels t-Test

ERGEBNISSE

PL-Gewinnung

Die Ergebnisse der PL-Gewinnung am lebenden Tier lassen sich folgendermaßen zusammen fassen

1 Die meisten PL-Proben enthalten eine gewisse Anzahl Erythrozyten Die Verwendbarkeit der PL hängt von der zu analysierenden Stoffklasse ab

2 Bei der Gewinnung von Gesamt PL durch Punktion des Knochens hatte ungefahr die Hälfte der Proben einen Blutgehalt ≤0,1 ‰ Diese Proben sind makroskopisch in der Ent nahmekapillare (Innendurchmesser 0,7+0,1 mm) farblos und klar

3 Bei der getrennten Entnahme der PL aus den beiden Schneckenskalen hatte offenbar wegen der zusätzlichen Perforation am Helikotrema, nur ungefähr ein Viertel der Proben einen Blutgehalt <0,1 ‰ Fur die Gewinnung der tympanalen PL ist die Punktion des runden Fensters gunstiger als die Punktion des Knochens

4 Durch Kautern läßt sich der Anteil der blutarmen Proben erhöhen Dabei tritt aber an der Kauterstelle, gemessen im Abstand von

Tabelle I *Proteinkonzentration (mg/100 ml) von mit unterschiedlicher Methodik postmortal gewonnener vestibulärer und tympanaler Perilymphe*

Probengewinnung	Vestibulare Perilymphe	Tympanale Perilymphe
Nach Perfusion mit Ringer Losung	177±56 (20)	177±61 (20)
Nach Perfusion mit Infukoll® M 40	133±36 (16)	150±28 (13)
Sofort postmortal	154±57 (27)	208±70 (15)

Bei einem Konzentrationsvergleich zwischen unterschiedlichen Versuchsreihen sind mogliche Unterschiede im Tiermaterial und in der Probengewinnung zu berucksichtigen

ungefähr 1 mm, eine mittlere Temperaturhöhung der PL von ungefahr 5°C auf und die Proteinkonzentration der Proben ist niedriger als bei Normaltieren

Bei der postmortalen PL-Gewinnung ist der Anteil der blutarmen Proben insgesamt größer als beim lebenden Tier und zwar bei der vestibularen PL in stärkerem Maße als bei der tympanalen Die von den intraarteriell perfundierten Tieren gewonnene PL ist nahezu blutfrei (Scheibe et al , 1972)

Proteinkonzentration von mit unterschiedlicher Methodik postmortal gewonnener PL

Wegen der größeren Schwierigkeit bei der intravitalen Gewinnung blutfreier PL und der damit verbundenen Problematik bei der Proteinbestimmung haben wir zuerst postmortal entnommene PL untersucht Die Proben wurden mit unterchiedlicher Methodik gewonnen

In Ergänzung unserer bisherigen qualitativen ntersuchungen (Scheibe et al , 1972) wurde zuıachst PL von mit Ringer-Losung intraarteriell ›erfundierten Tieren verwendet Begonnen vurde hier ebenfalls mit der Untersuchung der esamt PL Die mittlere Proteinkonzentration ›etrug 159±40 mg/100 ml ($n=37$) Ein Verleich zwischen den intraindividuellen Proteinerten des linken und rechten Ohres ergab bei en einzelnen Tieren zum Teil erhebliche Konentrationsunterschiede Die absolute Differenz zwischen der intraindividuellen Proteinkonzentration des linken und rechten Ohres betrug im Mittel 39±35 mg/100 ml Bildet man jedoch von den interindividuellen Proteinkonzentrationen den Mittelwert der linken und rechten Ohren getrennt, so ergibt sich kein signifikanter Unter schied, das heißt, die PL-Proben der linken und rechten Ohren gehören zur gleichen Grundgesamtheit

Danach wurde bei den mit Ringer Lösung perfundierten Tieren die PL aus den beiden Schneckenskalen getrennt untersucht Dabei war zwischen den vestibulären (177±56) und den tympanalen (177±61) Proben kein Unterschied der Proteinkonzentration nachweisbar (Tab I, 1 Reihe) Die Mittelwertdifferenz gegenüber der Gesamt-PL (159±40) ist nur zufällig

Um bei der intraarteriellen Perfusion der Tiere annähernd normale kolloidosmotische Bedingungen zu schaffen, wurde in einer anderen Versuchsreihe Infukoll® M 40 als Perfusionsmittel verwendet Die gefundenen Proteinwerte der vestibulären (133±36) und tympanalen Proben (150±28) sind in Tab I (2 Reihe) angegeben Die beiden Mittelwerte unterscheiden sich nicht signifikant ($P > 5\%$) Sie liegen etwas niedriger als bei der Perfusion mit Ringer Lösung Ein exakter quantitativer Vergleich der Mittelwerte ist jedoch grundsatzlich (Tiermaterial, Probengewinnung) nur innerhalb derselben Versuchsreihe zulässig

In einer weiteren Versuchsreihe wurde die PL ohne Perfusion der Tiere sofort postmortal entnommen Die erhaltenen vestibulären und tympanalen Proteinwerte sind ebenfalls in Tab I (3 Reihe) enthalten Hier liegt die Proteinkonzentration der tympanalen Proben (208±70) signifikant ($P < 1\%$) höher als bei den vestibularen Proben (154±57)

Proteinkonzentration intravital gewonnener PL

Ein Einfluß auf die Konzentration der PL durch die intraarterielle Perfusion der Tiere bzw die postmortale Probengewinnung ist zunachst nicht auszuschließen Wir haben deshalb auch am lebenden Tier gewonnene PL untersucht

Begonnen wurde zunachst ebenfalls mit der

Tabelle II *Proteinkonzentration (mg/100 ml) von mit unterschiedlicher Methodik intravital gewonnener vestibulärer und tympanaler Perilymphe*

Probengewinnung	Vestibuläre Perilymphe	Tympanale Perilymphe
Übliche Punktion	166±48 (44)	59±22 (33)
Liquorraum vorher subokzipital eroffnet	138±44 (19)	152±61 (29)
Zusatzlich subokzipital gewinnbaren Liquor aspiriert	144±34 (14)	126±58 (27)

Untersuchung der Gesamt-PL Dabei wurden jeweils Proben von ungefahr 6 μl bei einem Teil der Tiere von der vestibulären Seite, beim anderen Teil von der tympanalen Seite der PL-Raume entnommen Es zeigte sich, daß die Proteinkonzentration der tympanal gewonnenen Proben (83±25 mg/100 ml, $n=20$) signifikant ($P=1$ ‰) niedriger war als bei den vestibulär gewonnenen Proben (124±40 mg/100 ml)

In einer weiteren Reihe wurde die PL aus den beiden Schneckenskalen getrennt untersucht Dabei wurde die PL aus den Skalen so voll- g wie moglich entnommen Die Ergebnisse in Tab II (1 Reihe) dargestellt Daraus hervor, daß die Proteinkonzentration der aus der Skala tympani (59±22) nur ungefahr ein Drittel gegenuber der PL aus der Scala vestibuli (166±48) betragt Ein Links–Rechts–Vergleich zwischen den intraindividuellen Proteinwerten der einzelnen Tiere ergab ahnliche Konzentrationsunterschiede wie bei den perfundierten Tieren Die absolute Konzentrationsdifferenz zwischen linkem und rechtem Ohr beträgt im Mittel bei der vestibularen PL 44±26 mg/100 ml und bei den tympanalen Proben *24±19 mg/100 ml* Die Mittelwerte der interindividuellen Proteinkonzentrationen der linken und rechten Ohren unterscheiden sich jedoch auch hier nicht signifikant, weder vestibulär noch tympanal

Bei einem Vergleich mit den postmortalen Werten (Tab I) ergibt sich die Frage, ist die niedrige Proteinkonzentration der PL-Proben aus der Scala tympani real oder ist sie artifiziell bedingt? Bei der PL-Gewinnung am lebenden Tier sieht man, daß beim Eröffnen der Kochlea kontinuierlich PL aus der Punktionsstelle quillt Fur orientierende Messungen der austretenden Flussigkeitsmenge haben wir ausgezogene Entnahmekapillaren in die Punktionsstelle eingefuhrt und das Volumen pro Zeiteinheit bestimmt Im Verlauf der ersten 5–10 Minuten ergab sich ein Mittelwort von ungefahr 1 μl pro Minute Aus der punktierten Kochlea fließt dagegen keine PL, wenn der Liquorraum vorher subokzipital eröffnet wird Die gefundene Proteinkonzentration des Liquors beträgt 34±17 mg/100 ml ($n=15$) Es ist also anzunehmen, daß der niedrige tympanale Proteinwert Folge einer Mischung der tympanalen PL mit Liquor ist

Proteinkonzentration intravital gewonnener PL bei subokzipital eröffnetem Liquorraum

Um den Liquornachfluß in die Scala tympani beim Eroffnen der Kochlea weitgehend zu vermeiden, haben wir anschließend die PL bei subokzipital eröffnetem Liquorraum untersucht

In einer ersten Versuchsreihe wurden vor der PL-Gewinnung jeweils 5–10 μl Liquor entnommen Die Proteinwerte der untersuchten PL-Proben sind in Tab II (2 Reihe) angegeben Es zeigt sich, daß in diesem Fall die Proteinkonzentration der tympanalen PL (152±61) fast 3 mal so hoch ist wie bei nicht eröffnetem Liquorraum Der tympanale Mittelwert liegt sogar etwas höher als der vestibulare (138±44), unterscheidet sich aber von diesem nicht signifikant Die Einzelwerte der tympanalen Proben streuen relativ stark Bei ungefahr einem Drittel der Proben liegt die Proteinkonzentration uber 200 mg/100 ml, ein Teil der Werte liegt dagegen unter 100 mg/100 ml

*Möglicherweise kommt es auch bei subokzi*pital eröffnetem Liquorraum noch zu einem gewissen Liquornachfluß in die Scala tympani Wir haben deshalb in einer weiteren Versuchsreihe vor der PL-Gewinnung die gesamte subokzipital gewinnbare Liquormenge (ungefahr 50–100 μl) entnommen und durch etwas Luft ersetzt Der aspirierte Liquor war dabei häufig etwas blutig Die erhaltenen Proteinwerte der untersuchten PL-Proben sind ebenfalls in Tab II

(3. Reihe) enthalten. Auch bei dieser Versuchsreihe streuen die Einzelwerte der tympanalen Proben relativ stark. Der tympanale Mittelwert (126±58) ist sogar etwas niedriger als der vestibulare (144±34). Die Mittelwertdifferenz ist aber auch in diesem Fall nicht signifikant.

DISKUSSION DER ERGEBNISSE

Systematische Untersuchungen am Normaltier sind Voraussetzung für funktionelle Untersuchungen sowie für die Beurteilung pathologischer Befunde. Zur Gewinnung von Normalwerten werden gewöhnlich Proben analysiert, die am lebenden Tier unter möglichst physiologischen Bedingungen gewonnen werden. Die vorliegenden Untersuchungen zeigen aber, daß gerade bei der üblichen intravitalen PL-Gewinnung die Analysenwerte der tympanalen bzw. Gesamt-PL-Proben am stärksten artifiziell verfälscht sind. Die Proteinkonzentration der tympanalen Proben (59±22) beträgt in diesem Fall nur ungefähr ein Drittel von der vestibulärer Proben (166±48). Die geringe tympanale Konzentration wird offensichtlich durch Liquor verursacht, der beim Eröffnen der Kochlea hauptsächlich durch den Aquaeductus cochleae in die Scala tympani fließt (Proteinkonzentration des Liquors: 34±17). Diese Annahme wird durch folgende Befunde unterstützt:

1. Beim Eröffnen der Kochlea fließt am Anfang kontinuierlich PL (1 μl/Minute) aus der Punktionsstelle. Ähnliche Angaben haben Rauch (1964) und kürzlich Moscovitch et al. (1973) gemacht.

2. Bei subokzipital eröffnetem Liquorraum fließt dagegen keine PL aus der punktierten Kochlea und die Proteinkonzentration der tympanalen Proben steigt auf den vestibulären Wert an. Bei Katzen erhöht sich nach Verschluß des Aquaeductus cochleae die Proteinkonzentration der PL-Proben ebenfalls um das Zwei- bis Dreifache (Silverstein et al., 1969).

3. Morphologische (Arnold & v. Ilberg, 1971, Sando et al., 1971, Duvall & Sutherland, 1972, Jahnke, 1972, Moscovitch et al., 1973) und pharmakokinetische (Orsulakova & Stupp, 1972) Untersuchungen der letzten Jahre haben erneut bestätigt, daß der Aquaeductus cochleae beim Meerschweinchen auch für hochmolekulare und sogar korpuskulare Stoffe durchgängig ist. Intrazisternal appliziert erscheinen diese Stoffe nach kurzer Zeit (wenige Sekunden bis Minuten) zuerst in der Scala tympani der Basalwindung.

Es bleibt die Frage offen: Sind die bei eröffnetem Liquorraum gefundenen tympanalen Mittelwerte (152 bzw. 126 mg/100 ml) die wirkliche Proteinkonzentration in der Scala tympani oder ist diese sogar signifikant höher als in der Scala vestibuli, wie wir es bei den postmortal gewonnenen Proben (208 mg/100 ml) gefunden haben? Möglicherweise ist die relativ starke Streuung der tympanalen Proben bei subokzipital eröffnetem Liquorraum ein Hinweis, daß es auch unter diesen Entnahmebedingungen in einigen Fällen noch zu geringem Liquornachfluß in die Scala tympani kommt. Andererseits unterschieden sich bei den intraarteriell perfundierten Tieren die ebenfalls postmortal gewonnenen tympanalen und vestibulären Mittelwerte (Tab. II) nicht signifikant. Der höhere postmortale Mittelwert der tympanalen Proben (208 mg/100 ml) kann deshalb auch entnahmebedingt sein. Es ist daher wahrscheinlich, daß die Proteinkonzentration bei normalen unbeschallten Tieren auch in der Scala tympani im Mittel ungefähr 150 mg/100 ml beträgt, wie wir es bei allen vestibulären Proben, hier weitgehend unabhängig von der Art der PL-Gewinnung, gefunden haben.

In der Literatur liegen dagegen die meisten Proteinwerte der Meerschweinchen-PL relativ niedrig. Das ist wohl, abgesehen von unterschiedlichen Bestimmungsmethoden (Citron et al., 1956, Miyake, 1960), hauptsächlich auf eine Verunreinigung der Proben mit Liquor zurückzuführen. Rauch (1964) hat ohne Angabe experimenteller Details für die tympanale (58±16) und für die vestibulare (114±23) PL ähnlich unterschiedliche Proteinwerte mitgeteilt, wie wir sie bei der üblichen intravitalen PL-Punktion gefunden haben. Die übrigen Autoren haben die Proben ebenfalls intravital gewonnen und überwiegend nach Lowry et al. analysiert, aber

zwischen vestibulärer und tympanaler PL meist nicht differenziert Smith et al (1954) geben fur PL-Proben aus der Scala tympani einen Proteinwert von 50±17 mg/100 ml an Dieser Wert stimmt mit den von Rauch angegebenen *(58±16)* und unserem *artifiziellen tympanalen* Wert (59±22) uberein Rademacher (1966) fand bei 5 gepoolten Gesamt-PL von insgesamt *129 Ohren mit einer mittleren Blutverunreinigung* von 0,14 ‰ eine mittlere Proteinkonzentration von 75 mg/100 ml Auch hier durfte der *Zufluß von Liquor den Wert beeinflußt* haben Abweichend von diesen niedrigen Proteinwerten haben Schindler et al (1965) ohne Angaben zur Methodik fur PL eine Proteinkonzentration von 227±163 mg/100 ml und fur Liquor von ebenfalls 201±143 mg/100 ml mitgeteilt Schnieder (1968) hat fur PL sogar einen fast doppelt so hohen Mittelwert (400±171) angegeben Diese hohen Proteinwerte sind vermutlich durch Blutverunreinigung der Proben oder andere Artefakte bedingt

Daraus folgt, daß neben der *Blutverunreinigung* die Liquorverunreinigung tympanaler intravital gewonnener PL-Proben stärker als [illegible]er zu berucksichtigen ist In der Literatur [illegible]'e auf dieses Problem bei quantitativen PL-[illegible]suchungen am Meerschweinchen (Rauch, [illegible]964 Stecker & Cody, 1966) und an der Katze (Davies, 1968 Silverstein et al , 1969) bereits hingewiesen Diese Artefaktmoglichkeit ist bei allen biochemischen und physikalisch chemischen PL Untersuchungen zu berucksichtigen, bei denen die Kochlea eroffnet wird Eine Minderung dieses Unsicherheitsfaktors kann durch Eröff nung des Liquorraumes zur Druckentlastung erreicht werden

Besonders problematisch ist die PL-Analyse bei funktionellen Untersuchungen Hier können mögliche Konzentrationsanderungen in der PL durch den Liquornachfluß in die Scala tympani verwischt werden Eine weitere Schwierigkeit, mit der bei funktionellen Untersuchungen zu rechnen ist, lassen die bei einigen Versuchsreihen durchgeführten Links–Rechts–Vergleiche erkennen Die intraindividuellen Proteinwerte des linken und rechten Ohres differierten bei einzelnen Tieren erheblich Ein Maß für diesen Links–Rechts–Unterschied ist der berechnete Differenz-Mittelwert und dessen Standardabweichung Diese sog intraindividuelle Streuung liegt ungefahr in der Größenordnung der *interindividuellen Streuung* *Das* erhöht auch bei einseitigen funktionellen Untersuchungen (Paarvergleich) die Schwierigkeit, geringe Konzentrationsänderungen in der PL eindeutig festzu stellen

Die vorliegenden Ergebnisse zeigen, daß *systematische Untersuchungen unter weitgehender* Berucksichtigung der Fehlermöglichkeiten die unabdingbare Voraussetzung fur eindeutige analytische Aussagen sind Die als Zielstellung einleitend formulierten Fragen lassen sich folgender maßen beantworten

1 Die mittlere Proteinkonzentration der Meerschweinchen-PL beträgt, zumindest in der Scala vestibuli, ungefähr 150 mg/100 ml

2 Zwischen der Proteinkonzentration der vestibulären und tympanalen PL besteht wahrscheinlich *kein signifikanter Unterschied*

3 Bei der ublichen PL-Gewinnung am lebenden Tier wird die Proteinkonzentration der tympanalen bzw Gesamt-PL durch Liquor artifiziell bis auf ein Drittel erniedrigt Dies ist wahrscheinlich die Hauptursache fur die uberwiegend niedrigen Proteinwerte, die bisher von anderen Autoren angegeben wurden

SUMMARY

The protein concentration of the guinea pig perilymph was investigated systematically using a micro-modifica [illegible] of scala [illegible] *methods In living animals it is especially difficult* to obtain samples without blood contamination Another problem in the obtaining of perilymph from living animals is the contamination of tympanic perilymph samples with cerebrospinal fluid This contamination diminishes the protein concentration of perilymph to a high degree When the subarachnoid space is opened suboccipitally before perilymph extraction there is no significant difference between protein content in tympanic and vestibular perilymph The mean protein concentration in both cochlea scales is about 150 mg 100 ml When samples are extracted post mortem from animals perfused intra arterially mean values of *protein are in*

the same range Without perfusion of animals, the mean value of tympanic samples extracted post mortem is significantly higher Causes of artefacts in perilymph investigations are discussed

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ASPARTATE AND ALANINE TRANSAMINASES IN MIDDLE EAR EFFUSIONS

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Abstract Mucous secretion from 24 ears with secretory otitis media was analysed for aspartate aminotransferase (Asp AT) and from 18 ears for alanine aminotransferase (Ala AT) The activity of both these enzymes in middle ear fluids was 40 times larger than in serum, the differences being statistically significant The finding further corroborates the active secretory capacity of the proliferating middle ear mucosa

Transaminases constitute an important link between protein and carbohydrate metabolism and are widely distributed in tissues They occur particularly in heart, liver and kidney in tissue [illegible] ...tions that may be several thousand [illegible] larger than in serum These enzymes have [illegible] been studied from middle ear effusions with somewhat contradictory results Thus Paparella & Dito (1964) reported from an unspecified number of "adult male symptomatic ears" that in "several specimens" the Asp AT *(GOT) and Ala AT (GPT) had a range of activity* equivalent to normal serum Lupovich & Harkins (1972) determined Asp-AT from 201 specimens and found the values to be increased They expressed the activity values in Babson units which is identical to international units and obtained a range of 10 to 1 135, with an average of 335 Enzyme activity of serum was measured in 15 patients and the values were noted to be "within the normal range for serum for that pediatric age group"

MATERIAL AND METHODS

Middle ear fluid was aspirated during myringotomy in general anesthesia from 24 patients for Asp-AT and from 18 patients for Ala AT determinations The ear fluid had a mucoid character in all cases, mostly representing typical glue ears A sample of venous blood was drawn simultaneously for corresponding analyses of serum activity All patients were children

The total protein concentration of serum and middle ear effusions were determined using the method of Bramhall et al (1969) in the same way as Palva et al (1974) Quantitative analysis of Asp-AT and Ala-AT was carried out using Boehringer Mannheim GmbH test combinations, and the values obtained in mU/ml were converted to U/g of protein

RESULTS

The average total protein concentrations of serum and of middle ear effusions are shown for the series of Asp-AT determinations in Table I *The total protein value is clearly larger* in middle ear fluids than in serum but, due to the large spread of individual values the difference is only marginally significant ($p = 0.05$)

The Asp-AT enzyme activity figures expressed in units per 1 gram of protein are also shown in Table I The serum values show an average of 0 23 U/g with a small spread of results In middle ear fluids, the average activity is 40 times larger than in serum Even if the spread is considerable, the difference in activity figures is significant ($p < 0.01$)

Table II shows the corresponding results obtained in Ala AT determinations The average

Table I. *Comparison of total protein concentration and Asp-AT activity in serum and in middle ear fluids*

	Total protein g/l		Asp-AT U/protein g	
	Serum	Ear fluid	Serum	Ear fluid
' ı ($N=24$)	76 5	101 9	0 23	8 6
- D	11 8	67 1	0 05	12 3
Range	44 7– 110 2	27 9– 370 6	0 13– 0 32	0 9– 53 9

ıable II *Comparison of total protein concentra- and Ala-AT activity in serum and in middle fluids*

	Total protein g/l		Ala AT U/protein g	
	Serum	Ear fluid	Serum	Ear fluid
Mean ($N=18$)	84 6	82 9	0 12	4 8
D	12 9	39 5	0 04	5 9
,	58 7– 105 9	19 6– 153 8	0 06– 0 21	0 31– 21 4

otein concentrations are nearly the same in ı and in ear fluids Average activity figures lso for Ala-AT were about 40 times larger in ffusions than in serum, and the difference is ignificant ($p<0\ 01$)

DISCUSSION

ur data, showing a significantly larger Asp-ı and Ala-AT activity in middle ear effusions n in serum agree basically with the con- of Lupovich & Harkins (1972) However, their average Asp-AT activity in middle fluids is only about 1 5 times larger than the erum values as calculated from their normative ata, while the present results show activity 40 times larger in mucoid middle ear ids than in serum Simple filtration of these ymes through damaged capillaries is there- not possible, even allowing for consider- reabsorption of water from the fluid

This increase of Asp-AT and Ala-AT activity over serum is in line with that demonstrated earlier (Palva et al, 1974) for lactic and malic dehydrogenases and for acid phosphatase On the other hand as there was no increase in alkaline phosphatase, and the activity of esterases in serum was greater than in middle ear fluids, one must conclude that the middle ear mucosa secretes actively many enzymes to the effusion This agrees with the greatly increased secretory capacity of the middle ear mucosa after prolonged infection without drainage, manifested in the form of a large number of goblet cells and glands in histological sections (Friedmann, 1963)

ZUSAMMENFASSUNG

Die Aktivität von Asparagin (Asp-AT) und Alaninaminotransferasen (Ala AT) wurden an 24 bzw 18 Sekretproben aus sekretorischer Otitis media analysiert Die Aktivitat beider Enzyme war 40mal grosser im Mittelohrsekret als im Serum, und die Differenzen waren signifikant Die Resultate lassen vermuten, dass die proliferierende Mittelohrmukosa eine aktive Rolle in der Schleimbildung der sekretorischen Otitis media spielt

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SCHWINGUNGSMESSUNGEN AN VERSCHIEDENEN STEIGBÜGELPROTHESEN

Experimentelle Untersuchungen am menschlichen Schläfenbeinpräparat

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Abstrakt An menschlichen Schlafenbeinpraparaten wurden die Schwingungen der labyrinthären Flussigkeitssäule mit einem piezoelektrischen System bei verschiedenen Techniken der Otoskleroseoperation gemessen Verglichen mit der Amplitude bei normalem Steigbügel war bei den Operationsmethoden nach Zangemeister, Shea und Schuknecht ein beachtlicher Übertragungsverlust im Hochtonbereich festzustellen Tiefe Frequenzen wurden dagegen durchwegs fast genau so gut wie über den Steigbügel zum Innenohr fortgeleitet Oberhalb von 3000 Hz konnte der dampfende Einfluß des in das ovale Fenster eingesetzten Bindegewebes nachgewiesen werden Die Operationsverfahren nach *Zangemeister, Shea und Schu-* …nt ließen im Tief und Mitteltonbereich keine signi- … Unterschiede erkennen Lediglich eine wie ein … ston eingesetzte Robinson Prothese blieb in der … rtragungsleitung zuruck, während die gleiche Pro*these auf Bindegewebe aufgesetzt den anderen Verfahren* gleichzusetzen war

Die Steigbügeloperation ist heute, 20 Jahre nach dem ersten erfolgreichen Versuch der Stapesmobilisation durch S Rosen (1953), ein Routineeingriff geworden, der eine sehr hohe Erfolgsrate aufweist Allerdings wurden im Laufe der *Jahre verschiedene Methoden versucht, die* neben Dauererfolgen vor allem eine Vereinfachung des Eingriffes zum Ziele hatten, dies nicht zuletzt in der Absicht, umfangreiche Manipulationen bei offenem ovalem Fenster und damit eine übermäßige Traumatisierung des Innenohres zu vermeiden

Der Erfolg der Otoskleroseoperation ist neben der Wiederherstellung der Kettenfunktion in erster Linie von der Leistung des Innenohres abhängig In diesem Zusammenhang haben Faktoren, wie Einheilung, Umbau des implantierten Bindegewebes, Entzundungsvorgänge in der Nähe des ovalen Fensters oder die mechanische Verankerung der Prothese einen entscheidenden Einfluß auf das endgultige Hörvermögen

Während Einheilungsvorgänge kaum durch den Operateur beeinflußt werden können, erhebt sich die Frage, inwieweit eine gewählte Operationstechnik fur das Hörergebnis Bedeutung erlangt Da Schwingungsmessungen an Steigbügel*prothesen im menschlichen Schlafenbeinpräparat* grundsätzlich möglich sind, lag es nahe, solche Untersuchungen auf mehrere Techniken des Steigbügelersatzes auszudehnen und allfällige Unterschiede der Übertragungsleistung bei verschiedenen Frequenzen in dB auszumessen

Der experimentelle Schwingungsnachweis an der Prothese selbst ist im Gegensatz zum normalen Steigbugel, dessen Exkursionen bereits 1941 durch v Bekesy untersucht wurden, problematischer Das weiche Bindegewebe einer Schu*knecht-Prothese liefert im Gegensatz zur* Steigbugelfußplatte keinen eindeutig reproduzierbaren Bezugspunkt fur den Beschleunigungsaufnehmer und absorbiert überdies eine beträchtliche Schallenergie, wenn die *Koppelung* beispielsweise an ein starres piezoelektrisches System erfolgen soll

Nach zahlreichen orientierenden Vorversuchen haben wir daher schon in einer früheren Untersuchung über Schwingungen an einer Schuknecht-Prothese auf eine direkte Messung des Steigbugelersatzes verzichten müssen Außer-

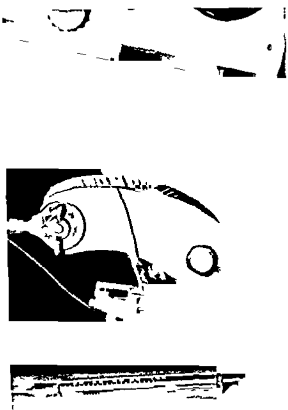


Abb 1 Das in eine Haltevorrichtung eingespannte Schläfenbeinpräparat An der Unterseite ist der Beschleunigungsaufnehmer mit dem Knochen in fester Verbindung Die Einstellung des Flussigkeitsspiegels ist mit der im Bild unten sichtbaren Spritze unter optischer Kontrolle durch das Mikroskop moglich

rdentlich brauchbar erwies sich dagegen der ˜chwingungsnachweis an der labyrinthären üssigkeitssäule Die Werte sind nicht nur gut eproduzierbar, sie entsprechen auch am ehesten en physiologischen Gegebenheiten

Wird die Flussigkeitsoberfläche an einem er- ›ffneten Labyrinthabschnitt mit einer Membran bgedeckt, die keine zu große Massenbelastung arstellt, ist die Untersuchung mit induktivem, apazitivem oder piezoelektrischem Beschleunigungsaufnehmer möglich, ohne daß ein direkter ontakt mit der Flussigkeitssaule erforderlich ird Bei entsprechendem flussigkeitsdichtem ufbau kann das Schlafenbeinpräparat in eine age gebracht werden, die ein Vorgehen wie bei iner normalen Steigbugeloperation gestattet, und es ist auch nach Eröffnung des ovalen Fensters nicht mit einem Ausfließen der Labyrinthflussigkeit zu rechnen (Abb 1) Wird dem ganzen System eine Kapillare angefügt, ist nicht nur ein Ausgleich des Flussigkeitsdruckes beim Zusammenfugen möglich, es kann auch der Flussigkeitsspiegel im ovalen Fenster gehoben oder gesenkt werden, wodurch ein optimaler Kontakt mit dem implantierten Bindegewebe oder Lappen unter dem Auflichtmikroskop gelingt

Fur den Schwingungsnachweis sind kapazitive Wandler in der Regel von Vorteil Da sie beruhrungsfrei arbeiten, entfällt die zusatzliche Massenbelastung praktisch zur Gänze Allerdings erfordern sie eine Mindestauslenkung, um einen genugend großen Frequenzhub—wie dies bei der direkten Modulation eines UKW Senders erforderlich ist—zu erzielen Wie Kobrak (1959) zeigen konnte, ist die Amplitude im Hochtonbereich gegenuber der Auslenkung bei tiefen Frequenzen wesentlich kleiner, und es bereitet die Registrierung sehr hoher Frequenzen mit einem kapazitiven Wandler größere Schwierigkeiten Da bei den beabsichtigten Vergleichsmessungen auch etwaige Veranderungen im Hochtonbereich festgestellt werden sollten, haben wir daher auf diese Meßtechnik verzichtet und ein piezoelektrisches System gewählt

Der Porus acusticus internus des menschlichen Schlafenbeinpräparates wurde nach Absägen der Pyramidenspitze auf 5 mm Weite aufgebohrt Nach Eroffnung des Vestibulums wurde ein Messingröhrchen eingeschoben und gegen die knocherne Wand mit aufgeschmolzenem Wachs abgedichtet Dann wurde das gesamte Hohlraumsystem mit Wasser gefullt und der Meßwandler auf das äußere Rohrende aufgeschoben Es war auf eine sehr kompakte Zusammenfugung mit massiven seitlichen Verstrebungen zu achten, um störende Eigenschwingungen (Schwingungsaufschaukelung uber die Knochenleitung) möglichst klein zu halten

Die Bearbeitung des Knochens erfolgte in tiefgefrorenem Zustand Durch die erstarte Lymphe konnte das Eindringen von Luft in die Bogengänge, was zu einem völligen Versagen der ge-

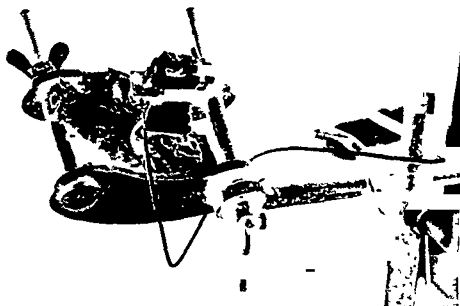


Abb 2 Das Schlafenbeinpraparat in der Ansicht von unten Die Pyramidenspitze ist abgesägt In den Porus acusticus internus ist ein Messingrohrchen eingeschoben Den Abschluß bildet eine quadratische durchbohrte Platte mit aufgeschweißter dunnster Styroflexfolie Die Verbindung zum piezoelektrischen System bildet ein im Mittelpunkt der Membran aufgesetzter Steg (im Bild als kleiner weißer Fleck innerhalb der quadratischen Platte sichtbar) Unterhalb, in einer Schlaufe dargestellt Kapil lare zum Druckausgleich Rechts im Bild geschirmtes Mikrophonkabel vom Beschleunigungsaufnehmer zum Verstärker

samten Versuchsanordnung gefuhrt hätte, ver- · · ι werden

Der für die Schwingungsmessung erforderliche ' wurde von einem Druckkammerlautsprecher uber einen Schlauch mit fest sitzender Olive dem äußeren Gehorgang des Schläfenbeines zugeleitet Ein angeschlossener Schallpegelmesser ermöglichte die kontinuierliche Messung der Lautstärke im äußeren Gehörgang Das piezoelektrische Signal des Beschleunigungsaufnehmers wurde nach Verstärkung auf zwei Arten registriert 1) durch oszillographische Aufzeichnung, und 2) durch Hörschwellenbestimmung im Kopfhörer (Abb 2)

Die zweite Untersuchungsmethode hatte gegenuber der ersten einen Vorteil Es konnte die notwendige Schallintensität sehr niedrig gehalten werden, sie lag durchschnittlich zwischen 50-70 dB Dabei war die von der klinischen Audiometrie her bekannte Fehlerbreite kein Nachteil, sie ließ sich erheblich einengen Der bei den Versuchen verwendete durchstimmbare Tongenerator ermoglichte im Gegensatz zur normalen Audiometrie mit fest eingestellten Frequenzen eine sehr genaue Festlegung der Hörschwelle durch gleichzeitige Frequenzverschiebung nach oben und unten neben der Betätigung des Lautstarkenreglers Wie zahlreiche Vergleichsmessungen ergaben, lag die Fehlergrenze nie höher als 2 dB Der Änderung in der Schallübertragung

Tabelle I *Schwellenanhebung* (+) *bzw -Herabsetzung* (−) *nach Vor- und Rückklappen des Trommelfelles in dB* (*Relation zur Situation vor der Operation*)

0 2	0 5	1 0	1 5	2 0	3 0	4 0 kHz
+3	−4	+4	0	+3	−2	
−4	−7	6	+3	4	12	−1
+1	−2	3	+2	6	5	0
0	+3	1	0	4	1	2
−4	+1	0	−1	0	3	
+7	+4	10	6	5	10	
0	−1	−2	1	0	0	0
+5	−1	−1	3	−2	5	4
0	+13	0	0	−4	0	−7
+1	+3	+5	+2	−3	0	0
DS +0 9	+2.1	+1	+1 2	−0 7	−0 2	+0 2

Tabelle II *Änderung der Schwingungsamplitude bei der Operation nach Zangemeister (Z) und Shea (S) gegenüber der Schuknecht-Prothese*

\+ = Anstieg − = Abfall in dB

0 2		0 5		1 0		1.5		2 0		3 0		4 0 kHz	
Z	S	Z	S	Z	S	Z	S	Z	S	Z	S	Z	S
−25	−27	−11	−11	−4	0	+2	+1	+3	−3	−10	+9	+6	−2
+14	+4	−21	24	−1	0	−1	0	−3	9	+2	+4	+8	+5
+14	+16	+7	+7	−5	+2	+3	+5	+3	0	+12	+10	?	?
0	+2	−1	0	−2	0	0	0	+2	+1	0	−3	?	?
−1	1	+7	−4	+1	12	+1	−1	−3	−2	0	0	−8	0
−4	−17	−5	17	+1	+7	0	0	−1	2	0	0	?	?
−9	−7	−6	−16	−10	−2	+2	−2	−2	+2	−3	3	?	?
	0		16		−10		0		−2		+5		?
+8	+6	+1	−2	+8	−6	13	−10	−12	−9	−5	2	−8	−3
+4	2	−6	4	8	−7	−15	−1	−12	−2	−12	0	2	−4
	+8		+3		+5		−2		−2		+2		−4
	3		−4		−4		−3		0		0		0
Ds +0 1	−1 8	−3 9	7.3	0 2	−2.3	−2.3	−0 4	−2.8	−2.3	−0 4	+1 8	−0 8	−0 8

von oft nur wenigen dB kommt bei den einzelen Meßvergleichen daher ein Aussagewert zu

Die Messungen wurden am intakten und ollig unberuhrten Trommelfell-Gehörknöchelhenapparat begonnen und das Ergebnis als *ezugswert fur die nachfolgenden Vergleiche* ierangezogen Die Wiederholung der Messung iach Vor- und Rückklappen des Trommelfelles ieferte beispielsweise einen Hinweis auf die ogliche Beeinflussung des Hörvermögens durch ie Manipulation am Trommelfell im Rahmen er Steigbugeloperation Wie aus Tabelle I erchtlich, sind Schwellenänderungen bis zu 10 dB iöglich, in den meisten Fällen waren sie aber ur so gering, daß sie als bedeutungslos betracht werden konnen Auch aus den Durchschnittserten ist auf keine ins Gewicht fallende Beeinussung der Schwingungsubertragung zu schlien Es heißt dies, daß die Spannung des Tromelfelles, die ja am Ende der Operation nach uruckklappen geringer sein muß, fur die Schallrtragung zumindest im Sprachfrequenzreich von eher untergeordneter Bedeutung ist bertragen auf die Verhältnisse in vivo heißt es, daß durch Einheilungsvorgänge im Tromlfellbereich nach Steigbugeloperationen nicht t einer Besserung des Hörvermögens zu rechn ist, wie dies gelegentlich behauptet wird

Die manchmal sogar gefundenen positiven Werte im Sinne einer „Verbesserung" der Schallübertragung sind nicht als Fehlmessung zu interpretieren Da es sich fast durchwegs um Schlafenbeine alter Menschen handelte, ist zu vermuten, daß die Rüttel- und Schiebebewegungen beim Abheben des *Trommelfelles* vom Knochen—wobei mit dem Separator bis zum Hammergriff vorgedrungen worden war—eine gewisse Lockerung der relativ steifen Gehörknochelchenkette bewirkt hatten (Wir gewannen bei den Präparationen den Eindrück, daß bei Schläfenbeinen älterer Menschen mit sehr hartem Knochen auch die Gehörknöchelchenkette stärker als ublich fixiert ist und nach Druck mit dem Instrument—wie bei einem Mobilisationsversuch—eine deutlich bessere Beweglichkeit besitzt Es ist vorstellbar, daß auf diese Art eine Verbesserung der Schalleitung zustandekommt)

Die Vergleichsmessungen erfolgten bei den drei bekannten Operationsmethoden nach Schuknecht (am langen Amboßschenkel festgeklemmter Stahldraht mit eingeknotetem, Bindegewebe im ovalen Fenster), Shea (Kunststoffröhrchen zwischen Amboß und Bindegewebe im ovalen Fenster) und Zangemeister (Bindegewebsinterposition zwischen Steigbügelschenkeln und ovalem Fenster), wobei mit der zuletzt erwahnten Technik begonnen wurde Das zwischen den Stapesschenkeln und dem ovalen Fenster inter-

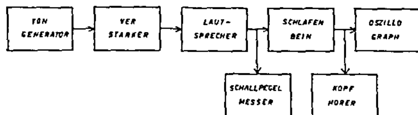


Abb 3 Prinzipschaltbild der Versuchsanordnung.

ponierte Bindegewebe diente dann auch als Auflage für das Kunststoffröhrchen nach Shea Ein etwa gleich großes Bindegewebsstuck verwendeten wir zuletzt für die Schuknecht Prothese Bei allen Verfahren wurde auf guten Kontakt mit der Labyrinthflussigkeit geachtet und der Wasserspiegel, wie bereits erwähnt, uber die Kapillare sorgfältig eingeregelt, damit im übrigen möglichst konstante Bedingungen erhalten blieben

Die tabellarische Zusammenstellung der einzelnen Messungen an 12 verschiedenen menschlichen Schlafenbeinen läßt beim Vergleich der Methode nach Schuknecht mit der von Zangemeister und Shea größere Unterschiede der Übertragungsleistung im Tieftonbereich erken- *(Tabelle II) Schwankungen bis zu 25 dB* [illegible] bei 200 und 500 Hz gemessen werden Es war allerdings nicht festzustellen, welche der Techniken die besten Übertragungseigenschaften besitzt Wie die Durchschnittswertermittlung zu erkennen gibt, scheint eine optimale Schwingungsubertragung nicht so sehr von einer bestimmten Technik abzuhangen, Beeinflussungen sind eher bei ein und derselben moglich, wenn das Implantat oder die Drahtschlinge in der Lage geringfugig verändert wird (z B Verschieben am langen Amboßschenkel)

Auch im Mittel- und Hochtonbereich sind die Übertragungseigenschaften der einzelnen Operationsverfahren nach diesen Vergleichsmessungen einander gleichwertig Es fiel allerdings auf, daß die Unterschiede zu den hoheren Frequenzen hin nicht mehr so deutlich hervortreten und nur ausnahmsweise Werte um 10 dB und daruber erreichen Diese eindeutig geringere Amplitudenänderung läßt auf eine verminderte Funktion in der Schallübertragung bei allen Verfahren im Hochtonbereich schließen Wir sehen darin eine Bestätigung der schon in einer fruheren Untersuchung gemachten Beobachtung an einer Schuknecht Prothese Die Übertragungsleistung nimmt im Hochtonbereich rasch ab, wobei der Unterschied zum normalen Steigbügel beträchtlich wird

Um die Kettenfunktion nach Steigbügeloperationen besser beurteilen zu können, haben wir die Amplituden bei den verschiedenen Operationsverfahren mit jener bei offenem ovalen Fenster verglichen (Abb 3) Die Meßapparatur war ausreichend empfindlich, um auch Tone, die die Labyrinthflussigkeit direkt in Schwingung setzten, bis in den Hochtonbereich registrieren zu können Dieser Ausgangswert entsprach dem direkten Schalleintritt durch das ovale Fenster *ohne Kettenfunktion und daher ohne Schall*drucktransformation Wie erwartet, stieg nach Einsetzen einer Steigbugelprothese die Amplitude der Flussigkeitsschwingung deutlich an und erreichte bei 500 und 1000 Hz Werte bis zu 27 dB Dieses Ergebnis entspricht genau der Schalldrucktransformation (siehe v Bekésy (1960) Experiments in Hearing) durch das Flachenverhaltnis des Trommelfelles zur Fußplatte (in diesem Fall zum wirksamen Querschnitt des Bindegewebsimplantates) und der Hebelubersetzung Hammer–Amboß Aus dem gemessenen Amplitudenanstieg ist zu folgern daß das gesamte System in diesem Frequenzbereich gut funktioniert und *in seiner Leistung dem nor*malen Steigbügel kaum nachsteht Die relativ geringen Werte bei Implantation einer Robinson Prothese sind nicht als typisch zu betrachten. Die ungleich schlechtere Übertragungsleistung ist vermutlich auf einen zu großen Spalt zwischen dem sehr dunnen Kolben und dem Perforationsrand der Fußplatte zuruckzufuhren Dadurch wird einerseits die Schwingung mit Energiever

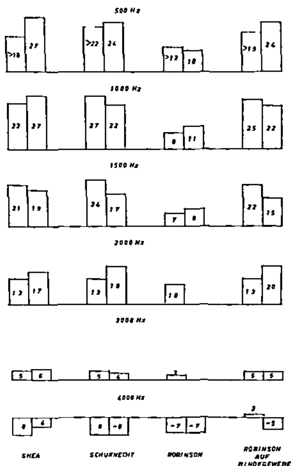


Abb 4 Übertragungsverbesserung ausgehend von der unterbrochenen Kette und offfenem ovalem Fenster (2 aparate)

(Ausweichphänomen) auf die Flussigkeits- le ubertragen, andererseits erreicht der Schall irekt und damit in anderer Phasenlage ungehin- ert das Labyrinth durch den Spalt zwischen Fußplatte und Prothesenkolben, eine negative Auswirkung ist ähnlich wie bei einer Perforation Trommelfell anzunehmen Wahrscheinlich urde ein dichterer Abschluß zwischen Kolben knöcherner Begrenzung durch Einheilungs- das endgultige Hörergebnis bessern

Wird die Prothesenschwingung bei höheren mit der bei offenem ovalem Fenster chen, so fällt auf, daß die Amplituden- fferenz schon ab 2 000 Hz deutlich kleiner ird und bei 3 000 Hz bereits unbedeutende erreicht Auch dieses Ergebnis entspricht fruheren Untersuchungen von Bekesy (1939), Wever et al (1948), nach deren Aussage die Schalldrucktransformation bis zu 2 000 Hz relativ frequenzunabhängig ist Die Prüfung der isometrischen Druckübertragung am Schläfenbeinpräparat ließ nach Bekesy einen steilen Abfall oberhalb von 2 000 Hz erkennen Es läßt dies den Schluß zu, daß bei hoheren Frequenzen ein zunehmend größerer Anteil der Kraft am Trommelfell zur Beschleunigung der Gehörknöchelchen in der erzwingenden Frequenz verwendet und im Hochtonbereich daher die Schwingungsenergie innerhalb der Kette fast vollständig aufgebraucht wird

Auf die Verhältnisse bei den verschiedenen Steigbugel-Prothesen übertragen, heißt dies, daß die über die Kette kommende Schallenergie nicht mehr ausreicht, das Implantat bei 3 000 Hz in eine so ausreichende Schwingung zu versetzen, daß ein Horschwellenanstieg eintritt

Oberhalb von 3 000 Hz sahen wir am Versuchspräparat nicht nur in keinem Fall mehr einen Amplitudenanstieg, sondern um 4 000 Hz sogar einen Abfall, d h , daß der uber das offene ovale Fenster eindringende Schall in der Intensität bereits wesentlich großer ist als jener uber die Kette und Prothese. Das Implantat besitzt somit in diesem Frequenzbereich eine dämpfende Eigenschaft

Um diese Annahme zu erhärten, wurde in mehreren Versuchen die Schuknecht-Prothese vom langen Amboßschenkel wieder getrennt, sonst aber in situ belassen es kam zu keiner Änderung der Amplitude, womit bewiesen war, daß der über die Kette kommende Ton für die Prothesenschwingung bedeutungslos geworden war Der gleiche Effekt war mit einem Bindegewebsstückchen ohne Drahtschlinge genau so auszulösen, es ist dies somit sicher nicht ein Prothesen spezifisches Phänomen, sondern steht in direkter Abhängigkeit von der Abdeckung des ovalen Fensters Die Amplitude stieg auch immer wieder an, wenn die Prothese oder das Bindegewebe aus dem Fenster entfernt wurde (Es ist in diesem Zusammenhang darauf hinzuweisen, daß die Phasendifferenz zwischen ovalem und rundem Fenster, die sich aus der 3–6 mm betragende Wegdifferenz herleitet (Kietz, 1960,

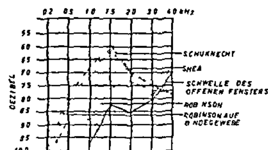


Abb 5 Der , Höranstieg bezogen auf das offene ovale Fenster

Lawrence, 1960) auf Grund der gegebenen Versuchsanordnung keine Berucksichtigung fand[1])

Höranstiege nach Otoskleroseoperationen werden nicht selten bis zu 8 000 Hz audiometrisch nachgewiesen, woraus klar hervorgeht, daß der Schalleitungsweg Trommelfell–Gehörknöchelchen–Innenohr auch im Hochtonbereich Bedeutung hat Eine Bestätigung dafur fanden wir in Versuchen uber die Schwingungsfähigkeit des Steigbugels fur sehr hohe Frequenzen (Cancura, 1969) Die Fähigkeit des Steigbugels, Schwingungen bis uber 10 000 Hz an das Innenohr zu ubertragen, zeigt, daß im Vergleich einer Steigbügel-Prothese *bei hohen Frequen-* grundsätzlich Unterschiede gegeben sind, ‚bung und Festigkeit der Fußplatte sind vermutlich dafur entscheidend

Was den Höranstieg bei Stapesoperationen im Hochtonbereich betrifft, so ist dieser nach den vorliegenden experimentellen Ergebnissen nicht direkt von der implantierten Prothese abhängig, wesentlich scheint vielmehr die Schaffung einer neuen Schalleintrittsoffnung durch Entfernung der mit dem Fenster starr verbundenen und daher schalldammenden Fußplatte zu sein So ist allein nach Entfernung der otosklerotischen Fußplatte bereits ein Horanstieg ohne Ketten funktion und somit ohne Schalldrucktransformation im Hochtonbereich zu erwarten Dieser Hörgewinn wird allerdings durch die unvermeidliche Implantation eines Bindegewebes mehr oder weniger herabgesetzt wovon zuletzt der end gültige Höranstieg abhängt

SUMMARY

In human temporal bone specimens the vibrations of the labyrinthine fluid were measured by a piezoelectric system after application of various techniques of otosclerosis surgery Compared with the amplitude in the

was almost equivalent to normal transmission via the stapes For frequencies of more than 3 000 Hertz the damping influence of the fibrous tissue implanted into the oval window was verified The results of surgery according to Zangemeister, Shea and Schuknecht did not differ significantly in the low and medium frequency range Only a Robinson prosthesis implanted like a Teflon piston caused an impairment of transmission while the method of fitting this prosthesis onto fibrous tissue was equivalent to the other techniques

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DEGENERATIVE CHANGES IN THE HUMAN VESTIBULAR SENSORY EPITHELIA

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Abstract A study of the vestibular end organs from humans of different ages is presented The inner ears were exposed by microdissection, and the vestibular sensory regions were either sectioned and studied with light or electron microscopy, or prepared and studied with the surface specimen technique A change, which can be related to aging, is the accumulation of lipofuscin inclusions in sensory and supporting cells, especially pronounced in the type I sensory cell Changes of the hair bundles, such as disarrangement of cilia, increased fragility of cilia and formation of giant cilia, have also been -d in aged individuals In three cases there was history of vestibular disturbance of vertigo All three had shown caloric hypo-reactivity In two cases ie with a history of herpes zoster oticus and another th a brain stem glioma no morphological changes hich could be attributed to the diseases, were found he third case showed degeneration of macula utriculi nd the lateral and superior cristae, possibly as a result f vascular disturbance

he conventional method of studying the nor- and pathologically altered human inner ear to examine serial sections of decalcified tem- bones This serial sectioning technique ves a satisfactory general picture of the mem- anous labyrinth, otic capsule, spiral and vestib- 'ar ganglia and vestibulocochlear nerve A oser investigation of the inner ear's sensory ons, especially in the vestibular apparatus, difficult, however, with this technique. For the subdivisions of the maculae, the iola and the pars interna and externa, cannot be discerned The distribution of the vestibular sensory cells, estimations of sensory cell populations and the investigation of the finer cyto-architecture of the neuro-epithelia are difficult or impossible to evaluate in material prepared with this method This is regrettable since vertigo is an important clinical symptom, and very little is known about its histopathological background It is therefore desirable to study the human vestibular end organs with other available techniques

Dizziness is found in many different ailing conditions *Not even in well known conditions*, such as Menière's disease, cerebellopontine angle tumors or viral infections of the inner ear, is the histopathological appearance of the vestibular sensory regions finally revealed Vertigo is also a very common complaint in elderly individuals Although central dysfunction, possibly caused by vascular insufficiency, might be a major factor responsible for vertigo in senescence, impaired function of the vestibular end organs might be another factor of importance

There are some observations indicating that the vestibular end organs are unaffected by aging (v Fieandt & Saxen, 1937, Schuknecht, 1955) A few reports describe a cochleo saccular degeneration, similar to that found in viral infections afflicting the inner ear, in aged individuals (Schuknecht et al, 1965, Johnsson, 1971) A moderate age-related degeneration of the maculae, and a more pronounced degeneration of the cristae have been reported by Rosenhall (1973)

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Correspondingly Bergström (1973) found a rarefaction of the vestibular nerve fibers of a similar magnitude in elderly individuals

Very little is known about the histopathology of the vestibular end organs in aged individuals Increased knowledge in this field is not only important to permit us to understand age-related functional changes of the inner ear, but also to be able to evaluate inner ear changes induced by a number of diseases, which cannot easily be duplicated in research animals Changes, such as a loss of cilia on sensory cells, accumulation of osmiophilic inclusions and the presence of laminated inclusions, have been attributed to Meniere's disease (Pietrantoni & Iurato, 1960, Friedmann et al , 1963) However, such findings can also be observed in vestibular sensory regions from non-Meniere cases (Hilding & House, 1964, Friedmann, 1967)

The ultrastructural effects of aging on the vestibular labyrinth have been investigated by Iurato (1967) He found that the sensory cells were irregular, and the nerve chalices around the type I hair cells appeared swollen and empty The hair cells were reduced in volume, including [illegible] the nucleus and the cytoplasm Electron-[illegible]se material was found in the space between [illegible] cell membrane and the nerve chalice The apical part of the sensory cell, including the sensory hairs and the cuticular plate, was fairly well preserved Laminated inclusions were found in the apical zone However, the author could not with accuracy decide whether all these morphological changes were typical for old age or not

MATERIAL AND METHODS

Originally the material consisted of inner ears from 96 "normal" individuals, ranging in age from the fetal period to 95 years of age In most cases (77) only the left ear was studied This material has been described elsewhere (Rosenhall, 1973) Six additional temporal bones have also been studied Thus the inner ears from three "normal" individuals were studied, as well as the temporal bones from three individuals who had suffered from diseases with possible influence on the inner ear In these cases clinical tests had indicated vestibular hypo-function

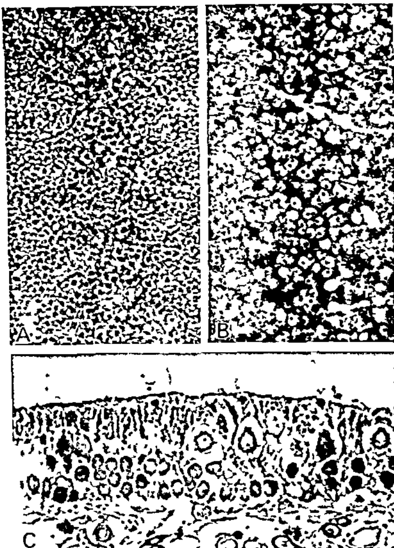
Each inner ear was fixed *in situ* (Bredberg, 1968, Rosenhall, 1972) with buffered osmic acid or with buffered glutaraldehyde These latter cases were postfixed with osmic acid The temporal bone was removed, and the labyrinth was rinsed with distilled water and preserved in alcohol The vestibular sensory regions were exposed by microdissection Most sensory epithelia were studied with the surface specimen technique, a procedure which has been described elsewhere (Lindeman, 1969, Rosenhall, 1972) Some end organs were embedded in Epon 812, and semi thin and thin sections were cut on a Sorvall Porter–Blum microtome or on an LKB Ultrotome The semi thin sections were stained with toluidine blue or with *p*-phenylenediamine and studied with light or phase contrast microscopy, using a Wild M 20 microscope Ultra structural studies were performed on specimens from three individuals Thin sections from these individuals were mounted on formvar-coated copper grids and stained with lead citrate and uranyl acetate and studied in a Siemens Elmiscope 1A Sensory epithelia from one individual were first studied with the surface technique specimen and then embedded in Epon and sectioned

RESULTS

Aging

When studying the gross anatomy it was observed that there were no pronounced alterations in size, configuration and thickness of the sensory epithelia in older individuals, compared with younger ones

Numerous round osmiophilic inclusions, measuring up to 3 μm in diameter, were found in the upper portion of the vestibular sensory epithelia from aged individuals They were not observed in fetal or infantile neuro-epithelia under the light microscope (Fig 1A) The inclusions occurred infrequently in specimens from young adults The number and the size of the dark bodies increased with increasing age, and they were abundant in both maculae and cristae from



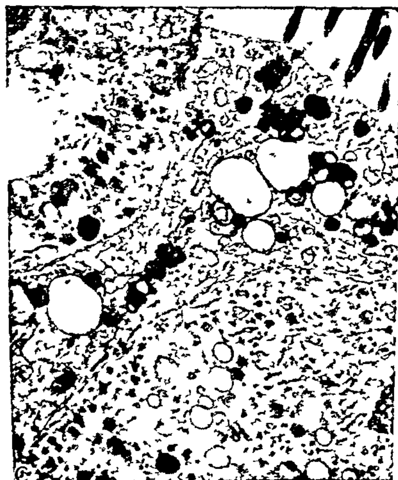
, *1* (A) Surface preparation of a fetal macula utriculi. The striola is seen in the center of the photomicrograph 170 (B) Surface preparation of macula utriculi from 74-year-old female There is a considerable accumula of osmiophil c inclus ons especially in the striola (dark stripe) × 150 (C) Section through the striola of macula utriculi from a 70-year-old male Numerous osmiophilic bodies are seen in the apical parts of the sensory and supporting cells × 560

viduals over 70 years of age (Figs 1B, C 2A) The inclusions were found in the supra nuclear part of the sensory cells (Figs 1C and B C) They were also observed in the upper ortion of the supporting cells The inclusions . . especially abundant in the type I hair cell , old individuals the macular striola appeared a dark stripe because of these inclusions (Fig ›) Autolysis or poor fixation did not have any iceable influence on the inclusions

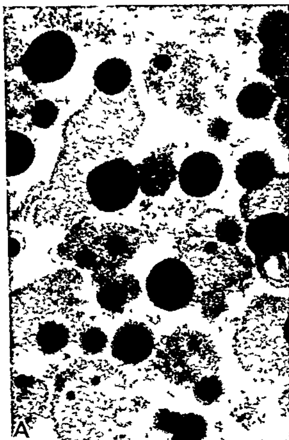
In the electron microscope it was observed that there were two different types of inclusions in the sensory cells and supporting cells (Fig 2C) In the sensory cells the inclusions were composed of an electron dense matrix, enclosed by a membrane The matrix contained numerous osmiophilic droplets with high electron opacity (Fig 2B, D) Vacuoles were also seen in the matrix These vacuoles were electron lucent or moderately electron dense (Fig 2B, D) The


A


B


C


D



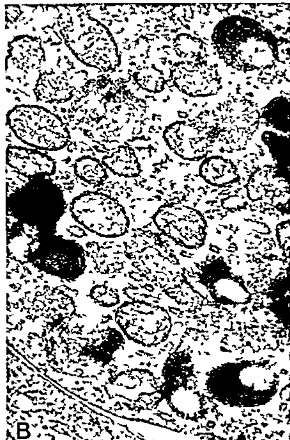


Fig 3 (A) Electron micrograph of the apical portion of a supporting cell from a crista from a 70-year-old male Numerous inclusions consisting of vacuoles embedded in granular masses are seen × 30 000 (B) Electron micrograph of the apical portion of a sensory cell from a posterior crista from a 4-month-old fetus. In the supra nuclear part of the cell numerous lysosomes and mitochondria are seen 40 000

lusions of the supporting cells consisted of round vesicles of different electron opacity (Figs C and 3A) The vesicles were surrounded by diffuse granular masses with no distinct limiting membrane

g 2 (A) Surface preparation of macula sacculi from 74-year-old female Numerous osmiophilic inclusions re seen in the sensory cells. 1500 (B) Electron micrograph of the upper portion of a type I hair cell from a rista from a 70-year-old male Inclusions consisting of of moderate electron density and dark droplets n a matrix, are seen 19 500 (C) Electron micro- i of a crista ampullaris from a 63 year-old male ne apical part of a type II hair cell is seen in the center the micrograph Numerous inclusions are seen in the asm The hair cell is surrounded by supporting lls which contain inclusions looking somewhat differ from those seen in the hair cell. 12 000 (D) In usions of a hair cell from the same crista as shown in Each inclusion consists of a matrix containing osmiophilic droplets and vacuoles. 65 000

Such osmiophilic inclusions were not found in fetuses However, in the fetal material lysosomes were often seen in the apical part of the sensory cells (Fig 3 B)

The hair bundles of the sensory cells showed distinct changes in old individuals Patches of the neuro-epithelia from individuals over 70 years of age were often more or less denuded of their sensory hairs (Fig 5A) In some instances only one long cilium, probably the kinocilium, remained on the hair cell while most of the stereocilia had vanished (Fig 4A) This change of the hair bundles was commonly seen in the cristae ampullares, while the maculae were affected to a lesser degree

It is important to determine whether this modification of the hair bundles occurred during the life, or if the cilia were torn away during the preparation For this reason a number of detached cupulae were placed in glycerine on glass slides and flattened by cover glasses Sensory hairs could be identified with phase contrast microscopy Cupulae from aged individuals contained large numbers of isolated cilia and even intact hair bundles, apparently detached from the underlying crista during the preparation (Fig 4C)

Because of the handling of the specimens during the fixation and microdissection the cupula on top of the ampullary crista was usually dislocated Some ampullae in which the cupulae had remained in normal position were embedded in Epon and sectioned The hair bundles of such cristae appeared normal under the light microscope (Figs 4B and 5B)

Disarrangement of the cilia in the hair bundles from inner ears of aged individuals was frequently seen (Fig 4A) Some of the hair bundles showed a more or less pronounced clustering of the cilia In some instances there was a complete fusion of the cilia and formation of giant ia Such giant cilia were only seen a few times neuro epithelia from older individuals (Fig D, E)

The age-related degeneration ultimately resulted in disappearance of sensory cells Before a hair cell disappears, it shrinks considerably and the free surface area decreases The sensory hairs disappear Finally, the hair cell vanishes and the supporting cells fill its place, and a scar is seen in the reticular lamina (Fig 6) Such scars are difficult to discern in human material, since the reticular lamina cannot usually be seen distinctly

Herpes zoster oticus (a case report)

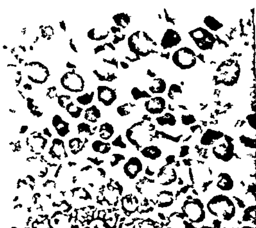
In 1961 an individual, at that time 72 years old, acquired a left-sided peripheral facial palsy He experienced no subjective hearing loss of vertigo Herpetic eruptions were found on the face and neck, and the diagnosis otic zoster was made The taste was slightly impaired on the left side of the tongue Pure tone audiometry showed bilateral high frequency loss Electronystagmography (ENG) revealed a right beating spontaneous nystagmus ENG was repeated 3 weeks after the onset of the symptoms There was no spontaneous nystagmus at this time The caloric test showed hypo-reactivity of the left ear

He died 8½ years later at the age of 81 of a carcinoma of the bile ducts

The left temporal bone was removed, and the sensory regions were studied All vestibular sensory epithelia were of apparently normal configuration and thickness (Fig 7A) Despite this, a considerable reduction of the sensory cell population was observed The maculae exhibited moderate reductions (20-30%) of the sensory cell populations (Fig 7B) A more pronounced degeneration was found in the cristae Between 40 and 50% of the hair cells were missing in the three cristae This degeneration was consistent with the age of the individual

Brain stem tumor (a case report)

This individual, a 28-year-old male, was first seen in 1968 He had noticed poor hearing in his left ear and difficulty in closing the left eye Pure tone audiometry disclosed total deafness of the left ear ENG showed a slight left beatings pontaneous nystagmus Caloric stimulation gave no response in the left ear Besides the symptoms from the eighth nerve, the patient had a facial paralysis on the left side, and he later developed other neurological symptoms X ray examination did not reveal any enlargement of the internal auditory meati A positive contrast cisternography, however, showed filling defects in both cerebellopontine angles An exploration of the brain stem was performed No extramedullary lesion of the eighth nerve was noticed The brain stem was much larger than normally seen, and the diagnosis brain stem glioma was made Irradiation with Cobalt 60 was initiated Altogether 4 000 rads were given to the brain stem The patient noticed some temporary improvement after the treatment He died in 1971 from the brain tumour





4 (A) Lateral cr sta from an 89 year old female ıs a cons derable d sarrangement of the c l a of ha r bundles In some ha r bundles sensory hairs are ng 1900 (B) Sect on parallel to the surface of a r or cr sta from a 76-year-old male The cupula is n the or g nal pos t on on the cr sta and the ha r bundles appear normal × 720 (C) The cupula from the superior ampulla of a 73 year-old female A large number of sensory ha rs detached from the cr sta are found n the lower port on of the cupula 1300


A


B


C
D


E



ig 6 Surface preparation of macula utriculi from a 76-r old male The arrow indicates the scar after a col lapsed sensory cell × 1900

The autopsy showed enlargement of the brain principally on the left side Histopathological studies showed a grade IV glioblastoma tiforme

Both temporal bones were removed and examined The vestibular neuro-epithelia were of normal size and configuration The hair cells and the supporting cells appeared normal under the microscope Quantitative analyses of both and of two cristae from each ear could carried out The sensory cell populations were normal, and no reductions of the number of hair cells were observed on either side

ig 5 (A, B) Transverse sections through cristae ampul from a 70-year-old male (A) and from a 68 year-old ale (B) The cupula has accidentally been removed the crista seen in A, while it still remains untouched the crista seen in B The crista without the cupula is of the sensory hairs, while the hair bundles normal in the preparation where the cupula is left × 320 B × 370 (C D) Giant hairs from cristae ampul- (C) Surface preparation from an 82 year-old female 000 (D) Surface preparation from an 81-year old le × 1900 (E) Electron micrograph showing a hair ndle from a 63 year-old individual One of the stereo- a appears considerably thicker than the others Notice kinocilium with 9+2 fibrils 26 500

Degeneration within the pars superior of the labyrinth (a case report)

This patient, a 77-year-old male, had a history of hypertension, which was treated with guanethidine (Ismelin®) and hydrochlorthiazide (Esidrex®) In 1965 at the age of 72 he experienced almost daily attacks of dizziness for a month Postural hypotension due to the hypertensive medicines was suspected However, ENG showed a reduced caloric response of the left ear No spontaneous, positional or gaze nystagmus was present, and the pure tone audiogram was normal

Five years later the patient died from cardiac failure The autopsy showed cardiac infarction and arteriosclerosis Arteriosclerotic plaques were found in the circle of Willis

The left temporal bone was removed and the vestibular apparatus examined The macula utriculi was atrophied The neuro-epithelium was thinner than normally seen and contained mainly supporting cells The between

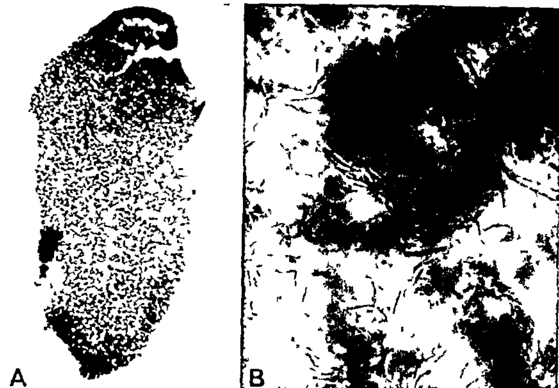


Fig 7 (A) Macula sacculi from the left ear from an 80-year-old male who had suffered from otic zoster The macula has normal shape and size *S*=striola ×37 (B) Surface preparation from the same macula The hair bundles appear normal and the degeneration is only slight ×1400

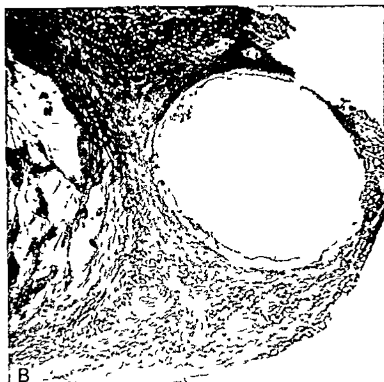
the macula and the surrounding perimacular ne were unsharp A few sensory cells remained the antero-lateral part of the macula The sensory epithelia of the lateral and superior cristae were thin and contained mainly cuboidal cells possibly supporting cells Only very few sensory cells could be identified scattered among these cells The border between the sensory epithelium and the planum semilunatum was blurred The walls of the superior ampulla were considerably thicker than normally seen Dense strands of fibrous tissue were connecting the ampulla and the adjacent part of the semicircular duct to the endosteum (Fig 8A B) This fibrous *tissue almost completely obliterated the peri* lymphatic space close to the ampulla

The macula sacculi and the posterior crista had normal size and shape and their cyto-architecture appeared normal under the microscope The hair cell populations of these sensory epithelia were recorded and were found normal for the age of the patient

DISCUSSION

It is commonly known that the cochlea under goes a continuous degeneration, increasing with age, but very little is known about the aging and the vestibular organ In 1973 Rosenhall showed that aging causes a reduction of the number of the vestibular hair cells and simultaneously Bergstrom found a rarefaction of the nerves running to the end organs Vestibular hypo-reactivity has also been reported in older individuals (Bruner & Norris, 1971 van der Laan, 1972)

Evaluations of hair cell populations of the *vestibular end organs, and of the number of* nerve fibers running to them, only indicate whether or not the neural elements are present Disappearance of hair cells and nerve fibers is preceded by more subtle degenerative processes afflicting the metabolism of the affected cells. Such modifications can be discerned with modern morphological technique



ig 8 (A) The superior ampulla and the adjacent part the semicircular duct from a 77 year-old individual th extensive degeneration within the pars superior of labyrinth (B) Section through the semicircular duct which is patent close to the ampulla The walls of the ampulla and the duct are thicker than normally seen and dense fibrous tissue is obliterating the perilymphatic space close to the ampulla A 15 B × 85

In the present study large inclusions were nd inside the vestibular sensory epithelia of ' individuals The electron microscopic ap earance of these inclusions is identical to the nce of neural lysosomes and lipofuscin (Samorajski et al, 1965, Koenig, 1969) uch inclusions have been described in inner ear y epithelia, and their relationship to lyso has been suggested (Ishii et al, 1967, om et al, 1970) Ishii et al (1967) showed th histochemical methods that these bodies lipofuscin inclusions, and they noticed that ie inclusions accumulated with increasing age milar osmiophilic inclusions have been de- ribed in cases with Menière s disease (Pietran- ni & Iurato, 1960, Ireland & Farkashidy, 1963, edmann et al, 1963) Such inclusions have been found in cases without this disease ng & House, 1964, Friedmann, 1967) y have also been found in the normal human of Corti (Kimura et al, 1964)

Electron microscopic studies of human ves tibular end organs have revealed a pronounced lack of sensory hairs (Pietrantoni & Iurato, 1960, Litton & Lawrence, 1961, Ireland & Far kashidy, 1963, Friedmann et al, 1963, Hilding & House, 1964) Remnants of both stereocilia and kinocilia were, however, often found Most of the specimen studied by these authors ema- nated from operated cases with Meniere's disease, and it has been suggested that this disease is responsible for the lack of cilia Hild ing & House (1964) proposed that the sensory cells could be denuded during the manipulation of the specimen, and they suggested that age might make human sensory hairs fragile

Loss of sensory hairs in the sensory regions from aged individuals is a prominent finding in the present study Patches of a sensory epithe- lium are provided with normal hair bundles while in other patches most of the cilia have disappeared In cristae with thinned hair bun-

dles, large numbers of cilia are often found in the cupula. Sections from cristae with the cupula intact on top have shown hair bundles with a normal appearance. The present study thus supports the opinion that the thinning of the hair bundles is a preparation artifact, as suggested by Hilding & House (1964). In young individuals the hair bundles are better preserved, which supports this supposition. The loss of sensory hairs might depend on increased fragility of the attachment of the stereocilia on the cuticular plate. Another possible explanation is that the aging cupula undergoes chemical changes causing the cilia to get stuck in the canals of the cupula.

Other alterations of hair bundles from aged individuals have been observed. Such changes are disarrangement and fusion of cilia and the presence of giant cilia.

Clumping of cilia and formation of giant cilia have been reported in the inner ear in a variety of pathological conditions (Duvall & Wersäll, 1964, Kimura et al., 1964, Ernstson et al., 1969, Winther, 1970, Bredberg et al., 1970 and 1972, Lim & Melnick, 1971, Stahle et al., 1973).

Histopathologic descriptions of inner ears affected by herpes infections are uncommon. Blackley et al. (1967) reported one case of herpes zoster oticus with facial palsy, tinnitus and deafness. In the vestibular labyrinth they found diffuse and perivascular round cell infiltration and degeneration of macula sacculi. Zajtchuk et al. (1972) described degeneration of the nerves supplying the superior and lateral crista in one case with a herpes infection. The case presented here had suffered from herpes zoster oticus with facial palsy and involvement of the vestibular system, as revealed by ENG. A long time, 8½ years, elapsed between the herpes zoster infection and death. The histologic study of the vestibular end organs revealed degenerative changes, including marked reduction of the hair cell populations. Such changes are normally seen in individuals of the same age as the patient of this study (Rosenhall, 1973). The degenerative changes observed in this case can thus be related to aging. Consequently, the viral infection did not cause any demonstrable permanent structural damage to the vestibular end organs.

Temporal bone histopathology of several cases with cerebellopontine angle tumours has been reported. No, or only moderate, pathologic changes of the vestibular end organs have been described (De Moura, 1967, Igarashi et al., 1971). In some cases degeneration of the vestibular nerve and ganglion with apparently normal end organs has been observed (Schuknecht, 1964, De Moura, 1967, Benitez et al., 1967). In one case with a meningeoma in the cerebellopontine angle Igarashi et al. (1971) found severe degeneration in the end organ apparatus. This degeneration was probably caused by vascular insufficiency.

In one of the present cases a brain stem glioma, expanding into the left cerebellopontine angle, produced severe audiological and otoneurological abnormalities. Similar clinical findings can be found in cases with acoustic neurinomas. The morphological study of the inner ear revealed apparently normal vestibular end organs. The reduced caloric response was obviously caused by disturbance central to the end organs, e.g. at the entrance of the eighth nerve into the brain stem or in the vestibular nuclei. This dysfunction at the brain stem level did not cause any conspicious degeneration peripherally at the level of the first neuron. It is also of interest to notice that the patient had received X-ray irradiation close to the inner ears. Such irradiation can cause degeneration of the inner ear sense organs (Winther, 1970). In the present case the irradiation did not cause any noticeable structural damage to the vestibular neuro-epithelia.

Cases of positional paroxysmal nystagmus where histopathological studies have revealed degeneration within the superior part of the labyrinth have been described (Lindsay & Hemenway, 1956, Cawthorne & Hallpike, 1957). Zajtchuk et al. (1972) described one case with herpes oticus, where degeneration of the superior and lateral ampullary nerves was present.

A case of degenerative changes of the macula utriculi and the lateral and superior cristae is

described here The macula sacculi and the posterior crista were of normal appearance The reason for this selective degeneration within the superior part of the labyrinth is nuclear, but occlusion of the anterior vestibular artery could be a possible explanation The patient had a consistent history of hypertension and cardiovascular disease

Fibrosis and new bone formation has been observed after hemorrhage into the perilymphatic space (Kimura & Perlman, 1956, Lindsay & Zajtchuk, 1970) and after laser irradiation (Stahle & Högberg, 1965) It is probable that the fibrosis of the superior ampulla and duct of the present case was caused by bleeding into the inner ear as part of a vascular disturbance involving the labyrinth

ZUSAMMENFASSUNG

ine Studie uber die vestibularen Sinnesorgane von Men chen verschiedenen Alters wird vorgelegt Die Innen- ohren wurden durch Mikrodissektion freigelegt Die estibularen Sinnesorgane wurden entweder geschnit en und mit dem Lichtmikroskop oder Elektronen nikroskop oder unter Anwendung der Oberflachen- oraparat technik studiert Eine Veränderung, die mit dem ltern zusammenhängen kann, ist eine Ansammlung on Lipofuscineinschlussen in den Sinnes- und Stutzzel n besonders ausgeprägt in Haarzellen des Typs 1 eranderungen der Haarbundel sowie Disorganisation er Zilien, vermehrte Bruchigkeit der Zilien und die ildung von Riesenhaaren wurden auch bei alten Perso- en beobachtet In drei Fallen wurde in der Anamnese uf vestibulare Krankheit oder Schwindel hingewiesen lle drei Falle zeigten kalorisch herabgesetzte Reizbar it In zwei Fallen der eine mit Herpes zoster oticus i der Anamnese der andere mit Gliom im Gehirnstamm, onnte man keine morphologischen Veranderungen nach eisen die von der Krankheiten herruhren konnten Der itte Fall zeigte Degeneration von Macula utriculi und on Cristae lateralis und superioris moglicherweise auf rund eines vaskularen Insults beruhend

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GLYCEROL EFFECTS ON THE PERILYMPHATIC AND CEREBRO-SPINAL FLUID PRESSURE

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Abstract Glycerol has been injected intravenously in guinea pig and its effects on the pressure in the cochlear fluids have been studied Simultaneously, arterial and cerebro-spinal pressures have been recorded Glycerol lowered the intracochlear as well as cerebro-spinal and blood pressures the latter only temporarily Different possible mechanisms for the glycerol effect on the intra cochlear pressure and its transient effect on hearing in Meniere s disease are discussed

In 1938 Hallpike & Cairns found a dilatation of the cochlear duct in two cases of Meniere's disease Since then histological examinations in approximately thirty more cases of this disease have shown a hydrops of the endolymphatic system It is now widely accepted that disturbances in the relations between the inner ear fluids cause the symptoms of Meniere's disease but the reason for these disturbances is not known

De Vincentiis et al (1964) reported a glycerol-dependent improvement of vertigo and hearing in two cases of "hydrops of the labyrinth" Klockhoff & Lindblom (1966, 1967, 1968) found that glycerol given to patients with Meniere's disease often causes a transient improvement in hearing and a decrease in tinnitus The improvement in speech audiometry was especially spectacular Since it was known earlier that glycerol lowers the intracochlear and the intracranial pressure it has been thought that glycerol may cause an improvement in hearing by reducing the pressure in the cochlear duct However, direct evidence for this pressure reduction has yet to be presented

The main purpose of this study has been to make direct observations on the variations of the pressures in the cochlea, in the cerebro-spinal space and in the vascular system after intra venous administration of glycerol

MATERIALS AND METHODS

25 young pigmented guinea pigs weighing between 280 and 400 grams were used Pressures in the cochlea, in the cerebro-spinal space and in the carotid artery were recorded, simultaneously when possible In 15 cases it was possible to make satisfactory recordings of intracochlear pressure throughout the experiment Blood pressure in 13 animals and cerebro-spinal pressure in 10 animals were recorded simultaneously

The animals were anaesthesized with intraperitoneally administered Urethane 1 33 g/kg bodyweight When needed, Lidocain was given locally The body temperatures of the animals were kept at a stable level by means of a servo regulating device

Tracheostomy was performed and the left external jugular vein and the left common carotid artery were cannulated

The atlanto-occipital membrane was exposed via the nuchal area A hole was made just big

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Table I *Simultaneous intracochlear, cerebro spinal and arterial pressures before and after glycerol injection*

S V – *scala vestibuli* S T = *scala tympani* S M = *scala media*

Animal no	Cochlear pressure cmH_2O					CSF pressure cmH_2O			Arterial pressure mmHg		
	Time (min)		0	10	20	0	10	20	0	10	20
	Peri lymph	*Endo-lymph*									
1	S V		6 0	4 0	–3 0	—	—	—	—	—	—
2	S V		7 0	3 0	3 5	—	—	—	—	—	—
3	S V		8 0	3 0	2 5	—	—	—	35	36	36
4	S V		5 0	4 0	0	—	—	—	35	38	39
7	S T		5 5	4 0	4 0	7 5	6 5	5 5	43	45	47
12	S V		6 0	4 5	4 5	12.0	7 0	5 5	27	33	27
13	S V		5 0	2.0	2.0	3 0	0 0	0 0	45	48	45
14	S V		10 0	7 5	7 5	3 0	0 0	0 0	34	54	40
16	S V		6 5	2 5	3 0	12 5	8 0	9 0	35	42	46
17	S T		3 0	0 0	0 0	2.0	–1 5	–1 0	43	48	51
18		S M	6 5	2 0	0 0	6 0	3 0	0 5	48	48	52
20		S M	8 0	6 5	5 0	8 0	5 0	5 0	50	55	52
’1		S M	6 5	3 0	2 5	9 5	6 0	6 0	67	61	65
’		S M	5 0	1 0	1 0	7 5	5 0	5 0	40	60	53
5	S T		5 5	4 0	4 0	—	—	—	40	43	44
lean			6 3	3 4	2 4	7 1	3 8	3 4	42	47	46
S D			+1 7 *n* 15	+2.0 *n*=15	±2 6 *n* 15	+3 9 *n* 10	+3 5 *n*=10	+3 5 *n*=10	+10 0 *n* 13	±8 5 *n*=13	+9 0 *n* 13

To test whether there was a statistically signifi ant difference between the cochlear and CSF ressures in the respective periods, the method f paired comparisons with the Student s *t* test as used for the 10 measurements where these ressures were recorded simultaneously The ean difference in the initial period was 0 9 cm H_2O ±3 7 S D, CSF pressure being slightly gher No statistical difference was noted

The triglyceride serum concentrations before and after glycerol injection are listed in Table II

DISCUSSION

In thorough studies Klockhoff & Lindblom (1966 1967 1968) were able to show that orally administered glycerol in many cases dra matically but temporarily improved the hearing

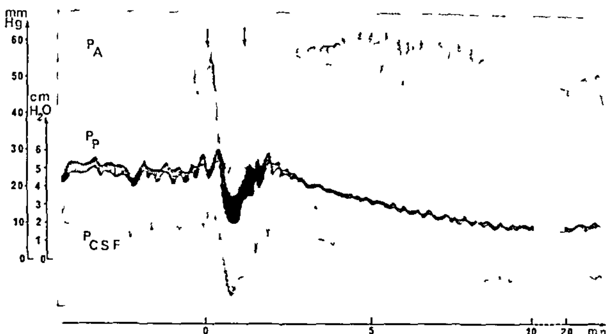


Fig 1 Recording of the effect of glycerol on the perilymphatic (P_P) cerebro-spinal (P_{CSF}) and arterial (P_A) pressures Representative recordings from experiment No 13 The arrows indicate the beginning and the end of the glycerol injection

in Meniere's disease Similar results have been reported by De Vincentiis et al (1972) As pointed out in the introduction, the exact mechanism concerning the glycerol action on the inner ear is not clear It has been proposed that glycerol has an osmotic effect, reducing the fluid volume and the pressure in the inner ear fluids

That glycerol will reduce the cerebro-spinal pressure has been shown by, among others, Cantore et al (1965), Meyer et al (1971) and Tourtelotte et al (1972) This lowering of the cerebro-spinal pressure is a plausible explanation for the headache which often occurs after glycerol administration This headache is known to vanish in the supine position

Crone (1965) was able to show the existence of a blood-brain barrier to glycerol which was confirmed by Waterhouse & Coxon (1970) Crone measured the arterio-venous difference in glycerol concentration and found that glycerol did not disappear more rapidly from the blood after passing through the brain vessels than did Evans blue which does not pass the blood brain barrier Waterhouse & Coxon found a low glycerol concentration in CSF and brain tissue after intravenous administration, much lower than in blood or muscle They interpreted this as being due to the existence of a blood-brain barrier The osmotic action of glycerol is probably the main cause of the reduction of the CSF pressure

Early studies on dog, cat and rabbit by Szasz (1926), Hughson (1932), Kobrak (1933–35) and Ahlen (1947) indicated that there is an intimate relationship between the pressure in the CSF and in the cochlea Hughson showed a higher pre in the CSF than in the cochlea Kerth & (1963) found equal pressure in the cochlea CSF in cat

Table II *Serum triglyceride concentration in mmol/l before and after intravenous injection of 2 4 ml/kg of 50 g % glycerol*

Animal no	Before injection	10 min after injection	20 min after injection
26	1 30	19 12	22 60
27	1 38	20 40	18 54
28	1 66	24 40	20 20
29	1 52	18 36	14 72
30	1 26	24 60	22 80

The relations between the pressures in the cochlea and in the CSF were studied by Martinez (1968) in guinea pig and cat and by Beentjes (1970, 1972) in cat Although both found the pressure in the CSF to be slightly higher, they reported a close interrelation between the cochlear fluids and CSF so that pressure variations in the CSF rapidly affected peri- and endolymph

The pressures in the cochlear duct and in perilymph in guinea pig were recorded by Weille et al (1958, 1961) and Martinez (1968) Their extensive experimental results indicate that the pressure is higher in the perilymph than in the endolymph In this respect Beentjes (1970, 1972) disagreed In the cat he found the pressures in peri- and endolymph to be equal

With equipment made especially for recording pressure in very small compartments, it has been possible to measure the intracochlear pressures with a high degree of accuracy There is no volume displacement in the recording system, so this permits recording of fast pressure changes

The risks of fluid escape through the opening made in the cochlear wall could not be eliminated but the rapid attainment of a stable pressure level after the hardening of the plastic indicates that the possible loss of cochlear fluid was quickly compensated for It is doubtful that it influenced the qualitative pressure changes

It was possible to record the pressure in the cochlear duct in 4 animals Although the lateral wall of the cochlear duct was carefully identified and the pipette was introduced using the micromanipulator, the recordings in the cochlear duct must be looked upon with some reservations Without a simultaneous recording of the endo- ochlear potential, it is not possible to be sure of that the pipette is in the right scala and that the cochlear duct is not damaged (Weille et al, 1958, McCabe & Wolsk, 1961, Beentjes, 1970, Hinojosa, 1971)

The results indicate that the pressures in perilymph and endolymph are identical under the ions used This is in agreement with the observations of Beentjes (1970) while it is in on to the results of Weille et al (1958, 961) and Martinez (1968)

A reduction of the intralabyrinthine hydrops has been suggested to cause the improvement in hearing after glycerol administration to Meniere patients (Klockhoff & Lindblom, 1966, Angelborg et al, 1971) In support of the theory that glycerol causes a fluid reduction in the inner ear are the observations of De Vincentiis et al (1972), who found a collapse of the cochlear walls and the semicircular ducts in guinea pigs after oral administration of glycerol

It has been possible to make simultaneous recordings of the pressures in the cochlea and in CSF in this present study and it has been shown that the cerebro-spinal pressure as well as the intracochlear pressure fall after intravenous injection of glycerol

Theoretically there are different ways in which the glycerol can bring about the lowering of the intracochlear pressure

1 The reduction of the intracochlear pressure depends upon the transient fall in the arterial pressure

2 There is a direct action of glycerol on the fluid secretion in the cochlea, reducing the production of inner ear fluids

3 The lowering of the intracochlear pressure is a result of the reduction of the CSF-pressure and depends upon a communication between the CSF and cochlea, mainly via the perilymphatic duct

4 Glycerol has a direct osmotic effect on the cochlea as it has on the CSF

The fall in the arterial pressure is probably not responsible for the reduction of inner ear and intracranial pressures The transient reduction in the arterial blood pressure after giving 50 % glycerol intravascularly has been noted before by Sloviter (1958) He examined various possible causes for this glycerol effect on the blood pressure and concluded that it was a peripheral vasodilating effect He did not find that it caused any persisting symptoms

It is doubtful that glycerol has a metabolic effect on the secretory functions in the cochlea since no glycerol effects on other secretory mechanisms have been reported Furthermore

glycerol is a non-toxic substance and its pressure-reducing effect starts too rapidly after glycerol administration

An osmotic effect of glycerol (Waterhouse & Coxon, 1970) is more probable The triglyceride concentrations 10 and 20 minutes after glycerol injection explain its long lasting effect on the cochlear and CSF pressures

A secondary pressure fall in the cochlea due to a drainage to the cerebro-spinal space demands a wide communication since the two pressures change simultaneously This is possible since the volumes in the inner ear are small and the cochlear aqueduct in the guinea pig is relatively wide However, this explanation is contradicted by the difference in the pressures recorded in the cochlea and in the CSF in this and in other investigations (Hughson, 1932, Martinez, 1968, Beentjes, 1970) In the present study there was a higher pressure in the inner ear in some animals, while in other animals there was a higher pressure in the cerebro-spinal fluid This indicates the possibility of individual and temporary variations When pressure recordings were carried on after sacrificing the animal, the ebro-spinal pressure invariably dropped to within 5–10 minutes, while the intracochlear pressure always rose This should not have been the case if there were a wide communication between the two different spaces, unless the communication rapidly deteriorated after death Therefore, a direct osmotic action of glycerol on the cochlear fluid seems probable

Various other substances were tested, as well as re injection of glycerol after the glycerol effect had been studied A rise in the pressure was noted after injection of water, saline, albumin and dextran The elevations were more or less temporary and no secondary pressure reduction was noted Re injection of glycerol caused the same pressure changes as the primary injection It is not advisable to draw conclusions concerning the effects of other drugs on the intra cochlear pressures when glycerol has been injected a short time before However, it is worth mentioning that repeated glycerol administration will cause pressure reactions similar to the first one, that these reactions are not noticed with the other substances and that it is possible to restore glycerol induced pressure reductions in the cochlea and intracranially

Glycerol intravenously administered to guinea pigs has been shown to reduce both the cerebro spinal and the intracochlear pressures Since glycerol given to humans will lower the cerebro-spinal pressure, it is most likely that it will also reduce the intracochlear pressure in a similar way However, the often favourable effect of glycerol on the hearing in patients with Meniere s disease may not be directly caused by this pressure reduction Glycerol may as well have another effect, perhaps a metabolic one on the cells in the organ of Corti or stria vascularis

It is not yet known if intravenously administered glycerol will cause a prompt improvement of the hearing in Meniere's disease If so this would be a strong indication of an osmotic effect, whereas a longer interval between injection and effect speaks in favour of a metabolic one Another way to determine whether the favourable effect of glycerol on the hearing loss in Meniere's disease is caused by an osmotic or metabolic effect is to use other osmotic active substances Further investigations in this field are being carried out

ZUSAMMENFASSUNG

Glyzerin wurde intravenos zugefuhrt und seine Wirkung auf Innenohrendruck intrakraniellen Druck und Arteriendruck bei Meerschweinchen studiert

Glyzerin senkte allgemein den Druck wahrend der Beobachtungsperiode den arteriellen Druck jedoch nur vorubergehend

Verschiedene Mechanismen fur die Wirkung des Glyzerins auf den Innenohrendruck werden diskutiert sowie die Frage wie Glyzerin temporär das Gehor von Patienten mit den Meniereschen Krankheit verbessern kann

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PROJECTION OF THE VESTIBULAR NERVE TO THE S I ARM FIELD IN THE CEREBRAL CORTEX OF THE CAT

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Abstract Evoked cortical focal potentials from electrical vestibular nerve stimulation were recorded in the Pcd area in cats anaesthetized with Chloralose or Nembutal For comparison additional cortical projections were located for n rad superficialis and group Ia muscle afferents from n rad prof, n fibularis prof, n femuralis ramus muscularis and the motor nerve to the trapezoid muscle Surface positive potentials, which reversed to negativity in middle cortical layers were for vestibular nerve stimulation recorded in the S I forelimb field in a small area close to Pcd in the posterior medial part of the deep radial nerve projection field The location of this field is compared with the vestibulo-cortical projections described earlier for rodents squirrel monkey, and rhesus monkey The histology shows that the field was within cytoarchitectonic 3a area

A primary vestibulo-cortical projection in a circumscribed area at the anterior suprasylvian sulcus (ASSS) of the cat was first described by Walzl & Mountcastle (1949) This field is located posterior to the face zone of the first somatosensory field (SI), and just anterior to the auditory area The projection is principally contralateral, but small potentials were recorded ipsilaterally Latencies were 6-8 ms These observations have been repeatedly confirmed in the same species (Kempinski, 1951, Mickle & Ades, 1952, Andersson & Gernandt, 1954) An overlap of the vestibular auditory and somatosensory representation has frequently been reported (Mickle & Ades, 1952, 1954, Landgren et al, 1967*a*) This may partly be explained by the fact that surface positive cortical potentials can be recorded over much larger areas than corresponding negative field potentials in deep cortical layers, which represent the sources of neuronal activity Kornhuber et al (1964*a*, *b*) have recorded a few neurons in the cat's suprasylvian gyrus, which responded to vestibular, proprioceptive and visual stimuli This cortical area also contains a muscle afferent projection field (Landgren et al, 1967*b*) in addition to the vestibular representation

The parietal vestibular field in the rhesus monkey (Kornhuber et al, 1965, Fredrickson et al, 1966*a*, *b*) has been found within the tip of the intraparietal sulcus Cytoarchitectonically this vestibular field, situated close to area 2, is unique Vestibular neurons were related to the somatosensory system for most of them were activated by proprioceptive input mainly from joints (Schwarz & Fredrickson, 1971*a*), but also from skin afferents (Schwarz & Fredrickson 1971*b*) This bimodal activation pattern had previously been noted for units in the vestibular nuclei (Fredrickson et al, 1966*b*), as well as in the monkey's thalamus (Deecke et al, 1973 Schwarz et al, 1974)

Cortical projection of the vestibular nerve found in the SI forelimb area of guinea pig rabbit, and squirrel monkey (Ödkvist et al, 1973*a*, *b*, 1974), could not be correlated with the parietal vestibular field found in the cat and rhesus monkey In the squirrel monkey this vestibular field was located cyto-architectonically in area 3a (Ödkvist et al, 1973, 1974, in press) the most rostral part of S I, which

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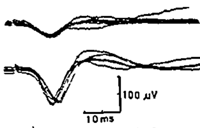


Fig 1a Surface positive responses in the Pcd vestibular field above and the response in the ASSS vestibular field below

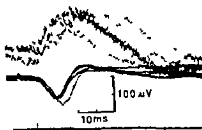


Fig 1b Depth reversal to negativity within the Pcd cortex above and the surface responses in the ASSS area below

receives muscle spindle afferents in different species (Oscarsson & Rosen, 1963, Phillips et al, 1971, Schwarz et al, 1973) A projection of the vestibular nerve to Pcd in the cat has been described by Sans et al (1970), and to motor cortex by Boisacq-Schepens (1971) and Boisacq-Schepens & Hanus (1972), but cytoarchitectonic verification was lacking as was comparison to muscle afferents projection fields

As the Pcd area in the cat has been thoroughly investigated concerning the projection of the group Ia forelimb and hindlimb muscle afferents Oscarsson & Rosen, 1963, 1966, Oscarsson et al, 1966, Landgren & Silfvenius, 1969, Silfvenius 1968) it seems important to correlate the vestibular projection field to the projection fields of the investigated extremity muscles

METHODS

Anaesthesia and general arrangements

Sixteen cats were used, eleven of which were aesthetized with 65 mg per kg body weight hloralose ip supplemented intermittently late in the experiment with accessory small doses Chloralose or with 0 I ml 3% Nembutal i v to reflex movements Five cats were in deep Nembutal anaesthesia Tracheostomy was routinely employed, and end tidal PCO_2 was recorded continuously in expired air If PCO_2 exceeded normal limits, the animal was placed on artificial respiration

In order to prevent brain oedema and fluctuations disturbing recordings, a suboccipital CSF leak was fashioned as well as bilateral pneumothorax The temperature of the animal was held between 37–38°C The animal was mounted in a stereotaxic apparatus I v glucose, penicillin and corticosteroids were administered in appropriate doses during the procedure

Preparations for electrical stimulation of the nerves

Bipolar or monopolar stimulating electrodes were implanted in the utricular and lateral ampullar nerves The nerves were exposed through the bulla They were reached after stapedectomy and destruction of a part of the basal turn of the cochlea Correct position of the electrodes was confirmed by observing the eye deviation produced by short train stimuli (50–100 Hz) In order to control current spread to neighbouring nerves, bipolar chlorided silver electrodes were also implanted in the facial and auditory nerves Electrodes for the cochlear nerve were inserted via a drill hole through the cochlea Stimulation intensities were kept so low that cortical evoked potentials could only be recorded within the primary projection field for each



Fig 2 To the left surface cortical responses to vestibular nerve and deep radial nerve stimulation for some representative points shown by the figures in the diagram *S A N S* ansate sulcus *S CRUC* cruciate sulcus, *S COP* coronary sulcus *ASSS* anterior suprasylvian sulcus *Pcd* postcruciate dimple

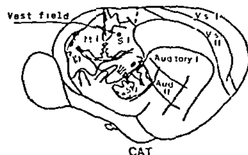


Fig 3 The vestibular field as compared to the SI field described by Woolsey (1964)

nerve Intensities resulting in a maximal cortical response for each nerve were usually below intensities which produced current spread to any of the neighbouring nerves

The following peripheral nerves were dissected and prepared for electrical stimulation deep radial nerve (DR), superficial radial nerve (SR), muscle branch of femoral nerve (FEM), deep fibular nerve (FIB), and one of the following nerves accessory nerve (ACC) or muscle branches of CII and CIII The nerves were cut peripherically, SR and DR mounted on bipolar chlorided silver wire electrodes under liquid paraffin for graded electrical stimulation The her nerves were engaged with a bipolar silver tunnel electrode, 0 1 ms square pulse was as stimulation, the frequency being kept at 0 5–1 Hz

Recording and mapping of cortical evoked potentials

Evoked cortical potentials were recorded from the surface of the cortex with a silver ball electrode, and from deeper cortical layers with a penetrating micropipette filled with 2 M NaCl and carried by a micro-manipulator in a stereotaxic instrument, see Fig 1 The projection fields were traced either on drawings of the cortex and its sulci and vessels or on the photographs of the cortex The border of the surface response field was defined as the point where the amplitude of the evoked potential had decreased to 30% of its maximum (Silfvenius, 1968)

Amplified potentials were displayed on cathode ray oscilloscopes and recorded by photographic superposition of multiple traces In some instances 25–50 sweeps were sampled by an averaging computer DIDAC 800

The recording sites were marked with electrolytic lesions or deposits of Pontamine Skye Blue The experiments were terminated by intravital perfusion with 10% formaldehyde The brains were mounted in paraffin and the recording sites identified on sagittal sections stained with cresyl violet

RESULTS

The zone where surface positive vestibular evoked potentials were recorded, was constantly positioned just anterior to the post-cruciate dimple The surface responses for some representative points on the cortex are shown in Fig. 2 The maximum positive potential was consistently found just anterior to Pcd In Fig 3 the vestibular field is compared to the cortical fields of Woolsey (1964) Starting from the maximum point depth recording was made with micropipettes, mapping the cortex in 200-300 μm steps, see Fig 4 The area giving depth reversal to negativity in middle cortical layers had a mean value of 1–2 mm²

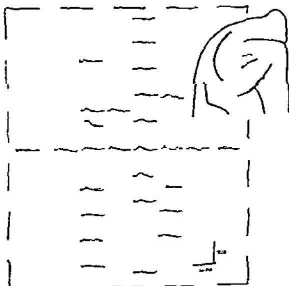


Fig 4 Within the box indicated with broken lines on the brain drawing and magnified to the left, are displayed the intracortical negative responses to vestibular nerve stimulation as mapped by a penetrating microelectrode

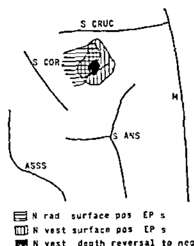
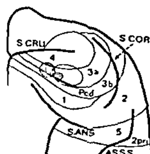


Fig 5 Cortical surface response to vestibular nerve stimulation (vertical hatching) area with depth reversal to negativity (blackened area) as compared to deep radial nerve responses cortical area (horizontal hatching) M midline

The cortical depth range within which the reversal to negativity was found varied somewhat, the values usually being between 500 and 1200 μm

The vestibular nerve surface positive and depth negative responses are compared to the deep radial nerve surface responses in Fig 5 The field with vestibular depth reversal to negativity was invariably within the surface field of he deep radial nerve In most cases we could even demonstrate points where depth reversal



g 6 The vestibular field marked by horizontal hatching visible within the cytoarchitectonic 3a area It is with the empty oval showing the projection of the deep dial nerve and it is not overlapping the group I projecn from the lower limb marked by vertical hatching (deeations from Hassler et al., 1964 and Silfvenius 1972)

Table I

Date	Exp no	Minim latency
10 07	1	4 3 ms
12/07	2	3 8
10 09	3	4 0
2/10	4	4.5
11/10	5	3 8
15/10	6	4 1
18/10	7	4 8
24/10	8	4 0
1/11	9	4 0
6/11	10	4 0
8/11	11	4 0
15/11	12	4 0
22/11	13	4 0
26/11	14	4 0
4/12	15	4 0
6/12	16	3 9
		$\bar{X}$ = 4 1 ms

was present both with vestibular nerve and deep radial nerve stimulation

The field with depth reversal to vestibular stimulation was never overlapping any other projection fields from the leg muscles or from the superficial radial nerve Nor did facial nerve stimulation or cochlear nerve stimulation give any responses in the Pcd-area

When the microelectrode was at a recording site yielding negative field potentials to vestibular nerve stimulation, as well as to deep radial nerve stimulation, it was investigated whether these potentials were caused by common neuron populations, by conditioning the vestibular stimulus with a radial nerve stimulus and vice versa The vestibular nerve stimulation did not have any detectable effect on the deep radial nerve field potential, nor did the deep radial nerve stimulation affect the vestibular response The delays used ranged from 15 to 300 ms In Fig 6 our vestibular field is compared to the cytoarchitectonic field as described by Hassler & Muhs-Clement (1964) and to the deep radial nerve representation as described by Silfvenius (1968) verified in our present experiments

The latencies were consistently short – close to 4 ms, see Table I From the surface of the projection area a remarkably synchronous spike-like potential could usually be recorded, probably representing arrival of spikes in thala

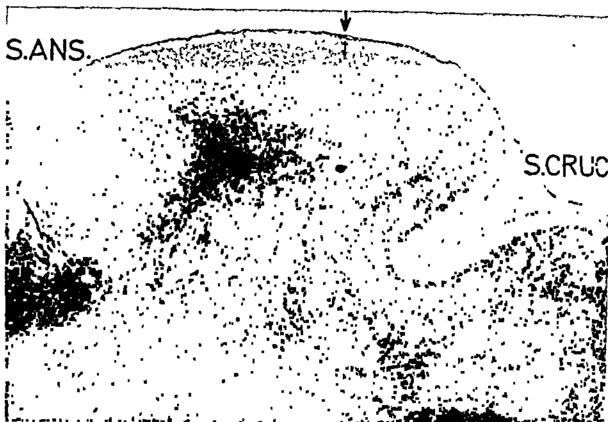


Fig 7 Histological specimen from experiment 26/11 showing a sagittal section through the recording site with track at arrow, at the end of which a deposition of Pontamine Skye Blue is made, the track clearly being in area 3a Cresyl violet

rtical fibres, which preceded a large cortical ential

The contralateral responses were invariably of greater amplitude than the responses ipsilateral to the stimulation site Latencies were not significantly different The histological evaluation shows that the vestibular field is within the cytoarchitectonic area 3a (see Fig 7)

DISCUSSION

The minimal latencies for the vestibular field potential were approximately 4 ms This is proof that the cortical area investigated is a primary vestibular projection field This 4 ms latency permits at least two synapses in midbrain structures, presumably one in the thalamus and one in the vestibular nuclei A short latency thalamic vestibular field has been described for the rhesus monkey by Deecke et al (1973) in the VPI nucleus. New data from the Saimiri sciureus (Liedgren & Schwarz, in preparation) justify the interpretation that thalamic vestibular cells projecting to both the parietal and the 3a field belong to the ventro-postero-lateral nucleus (VPL), which is the somatosensory nucleus This interpretation has become more reasonable in view of the fact that area 3a, containing the projection field described here, must also be regarded as part of the somatosensory cortex The vestibular cortical potentials that Fredrickson et al (1966*a*) described in the depth of the intraparietal sulcus of the rhesus monkey, have latencies of 5-6 ms. The vestibular evoked potentials described for other areas of the 'rhesus' cortex all have considerably longer latencies and were referred to as 'association areas' (Fredrickson, 1970)

Some vestibular projections outside the ASSS primary projection area in cats, which have

longer latencies should not be considered as primary fields as e g the vestibular projection in the cat motor cortex described by Boisacq-Schepens & Hanus (1972), the latency of which permits additional synapses Probably the ventrolateral (VL) nucleus of the thalamus (Raymond et al, 1974) and neocerebellar structures are involved in this projection or cortico-cortical connexions may participate The field described by Sans et al (1970) seems to be comparable to our field

For all animal brains the functional and cytoarchitectonic borders are more exact than the surface anatomical landmarks, i e the sulci It is therefore important to relate the position of the Pcd vestibular field to the well described and investigated muscle spindle areas for DR in the Pcd 3a area, (Oscarsson & Rosén, 1966, Oscarsson et al, 1966, Landgren et al, 1967*a*, *b*, Silfvenius, 1968) The consistent location of the vestibular field within the medial posterior part of this field places it in the map of well-established entities on the cat brain The fact that the lar field—albeit consistently of a smaller dium depth—always has one volume of true ield with depth reversal in common with the leep radial nerve field, gives it a defined three dimensional location The relation to projection areas of group I afferents of other motor nerves s also interesting The fibular nerve field is ial and not neighbouring the vestibular eld nor is the field for femoral nerve division the quadriceps muscle The facial nerve and accessory motor nerve or CII, CIII, did not any responses close to the dimple area

The lack of responses from facial nerve stimu on also proves that no spread of stimulation urrent to the facial nerve has occurred Further- cochlear nerve stimulation, electrical or d did not evoke any responses in this area

The position of this vestibular field within muscle spindle projection zones supports the ory that it is a field for integration of vesti and muscle impulses in balance, position, l motor control This could, however, only properly ascertained by a single unit analysis responses to both vestibular and muscle afferents The question also arises why the vestibular field is in the forelimb area and not in the leg area Functionally it is worth remembering, that the deep radial nerve innervates proximal extensors, i e antigravity muscles The fact that this field is located within the forelimb area and not within the hindlimb region does not permit the generalisation, that there is evaluation of vestibular impulses performed only in the forelimb somatosensory cortex In fact a recent study of the squirrel monkey's thalamus demonstrates that both fore- and hindlimb proprioceptors are integrated with vestibular receptors, although cortical field potentials can be recorded only in the 3a forelimb zone (Schwarz et al, 1974) The presence of a field potential indicates merely, that a great quantity of neurons fire simultaneously at this location Less densely packed active neurons at other locations can, of course, not be excluded by this study

The positions of this field in the cat falls well into the animal series Rodents have a vestibular field in the SI forelimb area, and in the squirrel monkey the position is the same (Ödkvist et al, 1973*a*, *b*, 1974, in press) For this species it has also clearly been shown that cytoarchitectonically the position is area 3a (Ödkvist et al, 1974, in press), the same position as now shown for the cat In the squirrel monkey brain this area reaches from the bottom of the central sulcus to the surface just anterior to it In the rhesus monkey, however, it is totally hidden in the depth of the central sulcus, which probably explains why it has evaded detection This explanation could also be relevant for the present lack of knowledge concerning the squirrel monkey parietal vestibular field, since it is probably situated deep within the Sylvian fissure Since lissencephalic rodent brains could not hide a projection field, it is probable that a parietal vestibular field does not exist in the 'lower' rodents

The cat is the only animal investigated that has both vestibular fields identified which is described above

An extrapolation from the animal series to man gives cause to suppose that, in the human

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A FLUORESCENCE AND ELECTRON MICROSCOPIC STUDY OF THE ADRENERGIC INNERVATION IN THE VESTIBULAR GANGLION AND SENSORY AREAS

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Abstract The adrenergic innervation in the vestibular ganglion and sensory areas in rabbit and cat was studied with fluorescence and electron microscopic techniques *All adrenergic nerve fibres consisted of postganglionic* axons from the ipsilateral superior cervical ganglion In rabbit postganglionic nerve fibres passed via the carotic plexus Probably because of the anatomical difference, the feline internal carotic artery being rudimentary, all sympathetic nerve fibres to the inner ear in cat passed via the tympanic plexus In the vestibular ganglion there was a uniform distribution of adrenergic nerve fibres and there was no difference in distribution patterns between rabbit and cat There was a continuous blood vessel innervation and an innervation independent of blood vessels The adrenergic innervation extended to the sensory areas but did not seem to penetrate the basement membrane and no adrenergic nerve terminals were found in direct contact with sensory or secretory cells

It is not known to what extent the vestibular part of the inner ear and its ganglion is supplied with an adrenergic innervation similar to that in the cochlea (Spoendlin & Lichtensteiger, 1966, 1967 Terayama et al , 1965, 1966, Densert & Flock 1974)

The possibility that the sympathetic nervous system may influence the inner ear under physiological and pathological conditions has often been considered (Lempert, 1946, Rambo et al , 1953, Beickert et al , 1956) An autonomic imbalance, i e sympathetic hyperactivity, has been said to cause neurovascular disturbances and various kinds of vertigo The effect of removing the sympathetic influence has been investigated, especially in cases of Men disease and tinnitus, following local anaest or surgical removal of the cervical ganglio tympanic plexus (Lempert, 1946, Passse, Lewis, 1954)

Lorente de Nó (1926) found that the va sensory areas were represented topographi in distinct portions of the vestibular ganglior he divided the ganglion into five areas recent studies (Gacek, 1969) divide the gan grossly in two parts—a superior division, re to the superior and horizontal canal cristae utricular macula and the antero-superior pa the saccular macula, and an inferior divi related to the posterior canal crista and saccular macula The numbers and course afferent and efferent nerve fibres have studied (Rasmussen & Gacek, 1958, Ross Cortesina, 1962) Electron microscopic stu (Wersäll, 1956, Spoendlin, 1965) showed number of non myelinated nerve fibres in vestibular nerve and even in the sensory a below the basement membrane Their or and character and whether or not they penet the basement membrane is not yet known

Various silver staining methods have b used to map out the sympathetic innervati Nerve fibres and even nerve endings of supp edly sympathetic origin were found in the tibular ganglion, the sensory areas and the me branous walls of the labyrinth (Bovero, 19 Palumbi, 1954, Andrzejewski, 1955, 195

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These fibres were traced to the superior cervical or stellate ganglion (Lorente de No, 1926) However, the methods used were non specific and the results were often contradictory With the aid of the histochemical fluorescence method developed by Falck et al (1962), the existence of adrenergic nerve fibres was observed by Fex et al (1965) Using the same histochemical method, Spoendlin & Lichtensteiger (1966) found adrenergic nerve fibres independent of blood vessels arranged in a terminal plexus below the vestibular sensory epthelium Their investigation was made on stretch preparations from macula utriculi and cristae ampullaris in the guinea pig

The aim of the present investigation was to study the sympathetic innervation of the vestibular ganglion and vestibular sensory areas in cat and rabbit at the cellular level, with a fluorescence method specific for catecholamines, and at the ultrastructural level, after administering a false transmitter to label adrenergic terminals

MATERIAL AND METHODS

25 cats and 21 white rabbits were used in this study

Fluorescence microscopy

In order to enhance fluorescence, most animals were injected with L-dopa (10 mg/kg i p) one hour before sacrifice The normal innervation pattern was studied in 2 cats and 5 rabbits To deplete the monoamine stores, reserpine (10 mg/kg i p) was administered to 2 cats and 2 rabbits 24 hours before sacrifice Under Nembutal® anaesthesia, the superior cervical ganglion was extirpated unilaterally in 3 cats and 2 rabbits In 4 cats and 4 rabbits the tympanic bulla was opened on one side and the tympanic plexus was cut Most nerve branches in the tympanic plexus passed over the promontorium In order to sever all nerve branches in the plexus and the auricular branch of the vagus nerve, part of the bulla adjacent to the temporal bone had to be removed The auricular branch of the vagus nerve was cut unilaterally in 3 cats and both the auricular branch of the vagus nerve and the ipsilateral tympanic plexus were cut in 2 cats

Animals were sacrificed under anaesthesia about 3 weeks after the operations The temporal bones were removed and the vestibular apparatus immediately dissected in cold Tyrode or Ringer solution The specimens were then freeze-dried and treated with formaldehyde gas according to the method of Falck & Hillarp (for ref see Corrodi & Jonsson, 1967) The specimens were embedded in paraffin and sectioned A Zeiss fluorescence microscope with a dark field condenser was used With this microscope the fluorescent products of noradrenalin appear green to yellow green and those from 5-HT and DA appear yellow Photomicrographs were taken using Scopi G film

Electron microscopy

Eight cats and 5 rabbits were pretreated with 5 OH DA (3,4 5 trihydroxyphenylethylamine), 4 × 20 mg/kg i p, over a period of 48 hours (Tranzer & Thoenen, 1967) In 1 cat and 1 rabbit unilateral cervical sympathectomy was performed about 3 weeks before pretreatment with 5 OH DA Two cats and 2 rabbits were not pretreated

The animals were anaesthetized with Nembutal i p or i v before sacrifice The vestibular parts of the inner ear were quickly dissected out and bone was removed to afford good contact with the fixation medium Fixation was performed in (*a*) sodium phosphate buffered 3 % glutaraldehyde at pH 7 3, and, after rinsing in Ringer, this was followed by postfixation in veronal acetate-buffered 1 % osmium tetroxide solution (Rhodin, 1954) (*b*) ice-cold 3 % potassium permanganate in 0 1 M sodium phosphate buffer at pH 7 0 for 45 minutes

After rinsing and final dissection in Ringer solution, the potassium permanganate-fixed specimens were contrast stained en bloc in ice-cold 1 % uranyl acetate in Ringer solution for 60 minutes (Richardson, 1966, Hokfelt & Jonsson, 1968 Hokfelt, 1968, 1969) The fixed specimens were dehydrated in ethanol and

embedded in Epon (Luft, 1961) Ultrathin sections were cut with glass or diamond knives on an LKB ultrotome

Sections from tetroxide-fixed tissue were contrast stained in uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), while sections from potassium permanganate-fixed specimens were only briefly contrast-stained in lead citrate Electron microscopy was performed with a Siemens Elmiskop I

RESULTS

Fluorescence Microscopy

In the fluorescence microscope, sympathetic nerve fibres in the vestibular ganglion and sensory areas showed a green or yellow-green fluorescence characteristic of noraldrenalin No indication for the presence of dopamine- or 5-hydroxy-tryptamine-containing nerve fibres or cell bodies was found in our specimens

Vestibular ganglion

Adrenergic nerve fibres were uniformly distributed throughout the ganglion and no part of the ganglion was devoid of adrenergic innervation

There was no essential difference in innervation between cat and rabbit, although rabbit had more adrenergic nerve fibres and occasionally several fibres were seen to course together, producing broad bands in which single varicosities were no longer discernible

The proximal part of the vestibular nerve contained only a few adrenergic nerve fibres and these appeared just before the ganglion cells The nerve fibres ramified and ran to different ganglion cells (Fig 1), travelling along the surface of the cells, where they exhibited distinct varicosities Other nerve fibres took a course independent of ganglion cells, some along blood vessels Between different groups of ganglion cells single fibres or bundles of adrenergic nerve fibres might be seen among myelinated nerve fibres, evidently independent of blood vessels

Arteries in the labyrinthine plexus had a rich adrenergic innervation but in the ganglion it was difficult to decide to what extent adrenergic nerve fibres were associated with blood vessels The ganglion cell cytoplasm contained numerous small particles producing a red-yellow autofluorescence



Fig 1 Fluorescent nerve fibres among ganglion cells in the vestibular ganglion Cat ×500

Sensory areas

Nerve bundles to the sensory areas contained a rather large number of adrenergic nerve fibres This was especially evident in the utricular nerve in rabbit Among myelinated nerve fibres, partly following blood vessels, partly independent of blood vessels, adrenergic nerve fibres were seen to approach the basilar membrane, but were not seen to penetrate it Fibres were abundant, but not to the same extent as in the plexus present in the habenula cochleae The saccular and ampullar nerves also contain adrenergic nerve fibres

In the ampullae and sacculus occasional fibr were seen under the basilar membrane but th



Fig 2 Section from the utriculus in rabbit fluorescent nerve fibres are found in abundance under the basal membrane ×200

never reached the sensory cells Epithelial cells in secretory areas contained a yellow autofluorescence which did not fade under ultraviolet illumination No adrenergic nerve fibres were seen in their vicinity The walls of the membranous labyrinth were examined but no evidence of adrenergic innervation or catecholamine-containing cell bodies was observed, neither was s the case at the base of the hair cells in any the vestibular epithelia where afferent and fferent fibres of the eighth nerve synapse

After cervical sympathectomy, all fluorescence aracteristic of catecholamines disappeared on the operated side both in cats and in rabbits The non operated side showed a normal distribution of adrenergic nerve fibres Cutting the ympanic plexus in rabbits did not produce any ffect on the eye, nor did it affect the innervation in the vestibular ganglion The same in cats gave a paralysed nictitans membrane and myosis on the operated side In the vestibular ganglion the innervation pattern was normal or the number of adrenergic nerve fibres was slightly reduced Cutting of the auricular branch of the vagus nerve did not influence the innervation pattern However, *sectioning of both the auricular branch of the* vagus nerve and the tympanic plexus led to the disappearance of all adrenergic nerve fibres in the vestibular ganglion and sensory areas In the proximal part of the severed tympanic plexus there was an intense accumulation of catecholamines (Fig 3) Some nerve bundles were devoid of adrenergic fibres—these probably belonged to Jacobson s nerve At the peripheral end of the cut plexus there was a slight accumulation of catecholamines In the proximal part of the cut auricular branch of the vagus nerve there was no fluorescence but close to this nerve trunk a separate tract of adrenergic nerve

fibres was observed showing an intense fluorescence This explains why the auricular branch of the vagus nerve had to be cut in order to ensure section of all sympathetic nerve fibres in the tympanic plexus

Electron Microscopy

Potassium permanganate is a very strong fixative and for some purposes gives an inferior ultrastructural preservation compared with fixation in glutaraldehyde and osmium tetroxide The advantage of using potassium permanganate is that it readily reacts with biogenic monoamines and gives a precipitate which is easily visible in the electron microscope (Richardson, 1966, Hökfelt, 1969) After pretreatment with 5 OH-DA, adrenergic nerve fibre terminals exhibit characteristic vesicles with a dense core Most of the vesicles seen in our specimens were found to be 400–500 Å in diameter, some being as large as 1 000 Å The number of vesicles in each terminal varied—sometimes only one or two were seen per section whereas other terminals seemed to be filled with vesicles Fixation with 'utaraldehyde and osmium tetroxide gave a or preservation of structures After pre- with 5-OH-DA, adrenergic nerve nals were seen to contain vesicles almost filled with an osmiophilic material

Vestibular ganglion

The arrangement of ganglion cells and the development of myelin sheaths were more easily studied when the specimens were fixed in potassium permanganate The size of the bipolar ganglion cells varied considerably within any given area of the ganglion, large and small ganglion cells were seen lying side by side Ganglion cells differed in the arrangement and thickness of their myelin sheaths, some ganglion cells had a thick myelin sheath while others had a very loose arrangement of myelin, often with only a single fragmented Schwann cell

At the axon hillock there was only a single Schwann cell layer This was true for the proximal as well as for the distal end of the ganglion cells Non-myelinated ganglion cells surrounded



Fig 3 When the tympanic plexus is cut an intense accumulation of catecholamines occurs in some nerve bundles Nerve bundles where catecholamines do not accumulate are also present Cat ×75

by only a Schwann cell were occasionally encountered In electron microscopy, adrenergic nerve terminals were found throughout the ganglion, either as single terminals, or as rows of terminals with a thin interconnecting nerve fibre (Figs 4 and 5) They were often uncovered, but could also be partly or totally surrounded by a Schwann cell There was no difference in the innervation to the different types of ganglion cell Adrenergic terminals were also encountered at the axon hillock Along myelinated nerve fibres adrenergic nerve terminals were often seen in bands arranged in parallel Sometimes terminals were observed at the nodes of Ranvier (Fig 6)

The ganglion was well vascularized, with blood vessels which were of capillary size and had no muscular component Adrenergic terminals were found close to endothelial cells (Fig 7)



Fig 4 Electron micrograph showing a row of adrenergic terminals (*NT*) along a vestibular ganglion cell (G) Fixation in potassium permanganate × 21 300

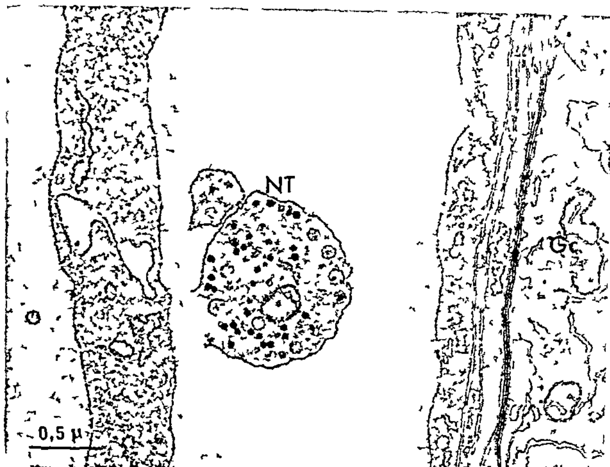


Fig 5 High magnification of an adrenergic nerve terminal (NT) close to a ganglion cell (Gc) Fixation in potassium permanganate × 35 000

Between a capillary and a ganglion cell a row of adrenergic nerve terminals could be seen where in one region a terminal faced the capillary and the other side was covered by a Schwann cell In neighbouring areas a nerve terminal could face the ganglion cell being separated from the capillary by the Schwann cell In association with blood vessels another type of terminal was also seen in a few places These terminals were filled with small clear vesicles and a few large vesicles containing a dense core

Sensory areas

In the branches of the vestibular nerve close to the sensory areas most adrenergic nerve terminals were found independent of blood vessels (Fig 8) Below the sensory epithelium adrenergic nerve terminals were seen They appeared as single fibres or sometimes travelled in bundles together with other non myelinated nerve fibres In electron microscopy the terminal plexus was not so well developed compared to the habenula plexus in the cochlea Adrenergic nerve fibres did not penetrate the basilar membrane and the absence of adrenergic terminals in contact with the sensory epithelium was confirmed Secretory regions in the ampullae and utriculus were examined but no nerve terminals were found in these areas and there was no evidence that secretory cells contained catecholamine granulae



Fig 6 In the vestibular ganglion adrenergic nerve terminals (*NT*) are sometimes observed at Ranvier's nodes Fixation in potassium permanganate ×26 500

DISCUSSION

The extent and possible influence of the sympathetic innervation of the inner ear has long been discussed Earlier morphological studies, which used non-specific methods and were limited to light microscopy, gave contradictory results Today it is possible, by using highly specific methods, to correlate light microscopic findings, e g fluorescence microscopy, with electron microscopic findings

In the present study the vestibular apparatus of cat and rabbit were investigated and compared

The sympathetic post ganglionic nerve fibres to the inner ear originate from the ipsilateral superior cervical ganglion Macroscopic dissection showed a definite difference between cat and rabbit concerning the course of the nerve fibres after they had left the superior cervical ganglion

After staining the post-ganglionic nerve fibres *in situ* with osmium tetroxide, the fibres in rabbit were traced to the internal carotic artery, giving rise to a plexus of adrenergic nerve fibres around this artery Only one small sympathetic nerve branch seemed to join a branch from the glossopharyngeal nerve which gives rise to the tympanic plexus in the middle ear Cutting the tympanic plexus in rabbit did not seem to influence the adrenergic innervation pattern of the inner ear In cat, however, all post-ganglionic adrenergic nerve fibres seemed

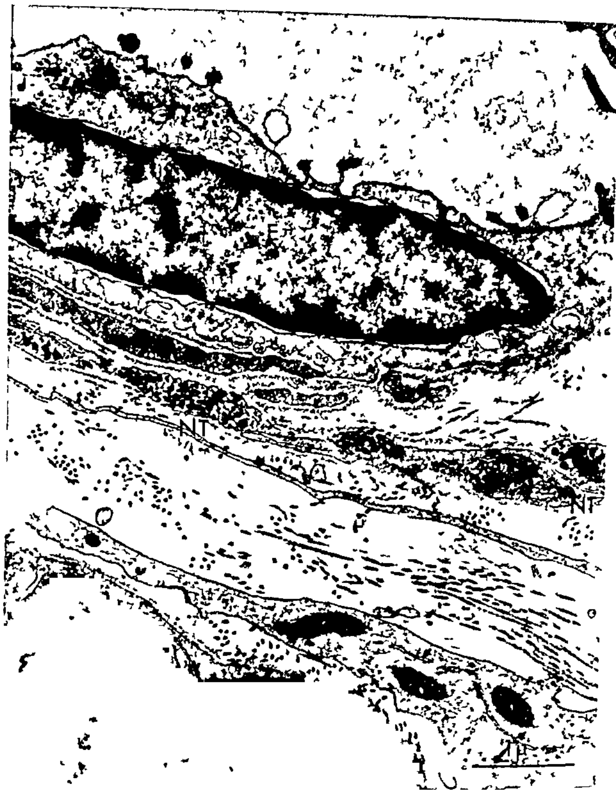


Fig 7 A cap llary in the vestibular ganglion with a row of adrenergic nerve terminals (*NT*) along an endothel al cell (*E*) Fixation in glutaraldehyde and osmium tetroxide ×26 500



Fig 8 In the nerve branches to the sensory areas adrenergic nerve terminals (*NT*) are found along myelinated nerve fibres (*MN*) Fixation in potassium permanganate. ×44 300

pass the middle ear, giving rise to a tympanic 'exus consisting of at least four or five macro-scopically identified nerve bundles Cutting the . nic plexus always resulted in paralysis of he Nictitans membrane but did not lead to rance of the vestibular adrenergic inner- It was found that on their way to the ear some adrenergic fibres run very close the auricular branch of the vagus nerve These fibres also had to be cut to assure total ympathectomy of the inner ear Fluorescence revealed an intense accumulation of atecholamines at the proximal end of the evered tympanic plexus nerves, but no adrener-ic nerve bundles were found in the auricular anch of the vagus nerve itself

In the vestibular part of the inner ear there is so a difference between the two animals in that the number of adrenergic nerve fibres is somewhat greater in the rabbit The difference, however, is not as pronounced as in the cochlea and the innervation pattern is in principle the same in the two animals

The vestibular ganglion has a richer innervation than the spiral ganglion Blood vessels in the vestibular ganglion and its sensory areas lack contractile elements In spite of this, they have a rather rich adrenergic innervation, with terminals lying close to the endothelial cells of the capillaries A possible adrenergic effect might be a diffuse influence on capillary permeability The labyrinthine arteries, however, have a muscular component and sympathetic stimulation might produce a vasoconstriction at this pre-capillary level Cholinergic innervation of the labyrinthine arteries has been demon-



Fig 7 A capillary in the vestibular ganglion with a row of adrenergic nerve terminals (*NT*) along an endothelial cell (*E*) Fixation in glutaraldehyde and osmium tetroxide ×26 500

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THE TRANSPORT OF RADIOACTIVE LABELLED SODIUM IONS AT THE ROUND WINDOW

Corroboration of the Mechanism of "Arslan's Operation" for morbus Meniere

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(Received April 22, 1974)

Abstract Radioactive-labelled sodium does not penetrate via the round window in the inner ear of guinea pigs. The findings reported confirm the mechanism assumed by Arslan for his "osmotic induction" surgery in the treatment of morbus Meniere

Most authors consider morbus Meniere to be a hydrops of the labyrinth, but the root cause is still hypothetical Numerous treatments aim at a reduction of pressure in the internal ear paces The range of possible treatments reaches m common dehydration of the inner ear, by infusions, to surgery of the organ (Arslan, 1969, 1970, 1971, Wilke & Richter, 1973)

Arslan describes a surgical method consisting in the deposition of crystals of sodium chloride in the niche of the round window By osmotic action the hydrops of the labyrinth is reduced and a normalizing of the biochemical and physiological surroundings is achieved

Subsequent to this surgical operation, Wilke & Richter (1973) found considerable deterioration of the inner ear hearing in 33% of the patients after 3-6 weeks Arslan (1971) describes the same phenomenon in 27% of his patients After the operation he observed an increase in the concentration of sodium ions in the endo- and perilymph of cats (Arslan, 1972) From the simultaneous depression and deformation of the microphonics, he deduced a disorder in the sensorial excitement of biomembranes as a result of changing ion concentrations

It remains to be clarified whether the described late damage is a result of such effects or not The salient question is therefore posed does the increase result from sodium ions by osmosis only, or does the exogenously applied sodium chloride pass through the membrane of the round window

A genuine increase in the absolute amount of sodium ions should have a stronger effect on bioelectric phenomena (depending on the corresponding sodium chloride concentration) than it has on the concentration changing by osmosis only

In order to elucidate this phenomenon we applied sodium chloride crystals labelled with ^{24}Na to the round window of guinea pigs and investigated the endo- and perilymph for radioactivity at various intervals

MATERIALS AND METHODS

In healthy adult guinea pigs weighing 400 to 500 grams, the round window was exposed in the auditory bulla after having made an incision beneath the auricle wall (Rauch, 1964) They were anesthetized intraperitoneally with ethyl-urethane A reflex to a sharp clap was seen in all of the animals before each test Radioactive labelled crystals of sodium chloride with activity

The authors wish to dedicate this article to Professor Moser on the occasion of his 65th birthday

Table I *Distribution of radioactive sodium ions*

The table shows the distribution of labelled sodium 24 in the biological material
Radioactivity was detectable in blood and urine only—endo- and perilymph contained none

Time h	Total applied amount (cpm)	Back-ground (cpm/ml)	Recovery (cpm/ml)	Material
2	32 000	48	54	Rinsing water
			42	Endo- and perilymph
2	35 000	48	62	Rinsing water
			40	Endo- and perilymph
9	30 000	48	58	Rinsing water
			48	Endo- and perilymph
24	46 800	60	50	Rinsing water
			53	Endo- and perilymph
24	45 000	60	59	Rinsing water
			54	Endo- and perilymph
48	25 300	55	46	Rinsing water
			63[a]	Faeces
			151	Blood
			50	Endo- and perilymph
			71	Urine
48	27 000	46	48	Rinsing water
			62[a]	Faeces
			348	Blood
			42	Endo- and perilymph
			69	Urine

[a] 500 mg

as shown in Table I were introduced into the round window and the wound closed again After intervals of 2, 9, 24 and 48 hours, the animals were decapitated under ether narcosis he treated inner ear was isolated, thoroughly vashed in rinsing water, and frozen in on the 'Frigomat" refrigerator equipment of Fa Jung, eidelberg The osseous capsule was scraped rom the cochlea by means of sharp milling hamond cutters under a stereo microscope of EB Carl Zeiss, Jena After that we could draw ff the frozen endo- and perilymph for measur-ng in test tubes In order to preclude smearing ver of radioactive materials preliminary to reezing, washing was necessary The water used and in some experiments blood and excrements 0) was tested for radioactivity The measure ients were performed in a scintillation-crystal etector with the counter VA-G-120 of VEB FT Meßelektronik, Dresden Due to the brief alf life of ^{24}Na, an amount of the labelled preparation equivalent to the sodium used in tests was reserved during the experimental period up to the moment of measurement It was measured simultaneously with the obtained biological material Thus the applied dose is related to the radioactivity existing at the moment of counting

RESULTS

The results of our investigations are shown in Table I It can be seen that no radioactive sodium has passed the round window into the internal ear The measured counts are within the range of the background In blood and urine, however, labelled sodium is detectable

DISCUSSION

Our results clearly verify no direct disturbance of biopotentials—such as microphonics taking place by exogenously applied sodium ions

which have passed into the inner ear The explanation concerning the mechanism of Arslan's surgical method, according to which the osmotic stimulation only restores the deranged biochemical and physiological balance of the inner ear, is corroborated by our results An osmotic impulse may be of importance for microcirculation within the labyrinth (Godlowsky, 1972) The same effect should theoretically be produced by compounds other than sodium chloride The question as to the causes of the severe hearing loss occurring in some patients 3–6 weeks postoperatively is nevertheless still unsolved It is safe to say that it is not an immediate effect of the concentration of sodium ions—otherwise it would be observed directly after the surgical operation Apparently the transport of sodium chloride out of the middle ear occurs very quickly, as shown by the measured values in the rinsing water Probably the main part passes via tube into the digestive tract, whence it is resorbed and excreted by the kidneys This is supported by the radioactivity found in blood and urine

Finally, a direct resorption via the mucous membranes of the middle ear may also be discussed Our time for investigation was limited the brief half-life of ^{24}Na, but in long-term riments no other results are to be expected, cause of the quick transport out of the middle ear

ZUSAMMENFASSUNG

Radioaktiv markierte Natriumionen sind nicht in der [illegible]

seiner osmotischen Entwasserung des Innenohres bei der chirurgischen Behandlung des Morbus Meniere

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A COMPARISON BETWEEN SUBJECTIVE AND OBJECTIVE RECORDING OF OCULAR COUNTER-ROLLING AS A RESULT OF TILTING

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(Received March 6 1974)

Abstract In order to develop a quick and reliable clinical od for recording counter rolling as an indicator the otolith function 14 normal persons were investi ed by both an objective and a subjective recording nethod For the objective recording a goniometer ocular a Zeiss microscope was used and for the subjective method a luminous cross manipulatable along the hree axes of the space The results show that the objective ing is much more reliable and better reproducible han the subjective and is consequently a good and recording method for clinical investigation of inter rolling.

Rotation of the eyes around their visual axis, o-called counter rolling, is obtained when the is tilted in the frontal plane to either side he reaction is considered to be elicited from otolith organs (Fluur & Siegborn, 1974)

Many different methods have been used for ng counter rolling They may be said, owever, to consist mainly of an objective and a ' The objective method, where rotation of the eyes is recorded by an , involves an observation of artificial natural landmarks on or in the eye Among e artificial landmarks may be mentioned the ttooing of a cross on the cornea (Benjamins, Krejcova et al, 1971), egg membrane e Kleyn & Versteegh, 1924), or gelatine mbrane on the cornea (Benjamins, 1926), silk threads sutured subconjunctivally raybiel & Woellner, 1959) To the objective ks belong corneal astigmatism (Nagel, 71), conjunctival vessels (Nagel, 1871) natural ots on the iris (Burow, 1841, Nagel, 1871, rany, 1911 Kompanejetz, 1923, Benjamins, 1926, Free & Jones, 1960, Miller, 1962, Miller & Graybiel, 1963, Hannen et al, 1966, Nelson & House, 1971) or the rotation of the retinal papilla (Grahe, 1938) The investigator records the alteration in position of these landmarks during tilting, either by direct visual inspection of the eye (Bürow, 1841, Nagel, 1871, Kompanejetz, 1923, de Kleyn & Versteegh, 1924, Benjamins, 1926, Grahe, 1938) or by photographic recording (Graybiel & Woellner, 1959, Free & Jones, 1960, Miller, 1962, Miller & Graybiel, 1963, Hannen et al, 1966, Krejcova, 1971, Nelson & House, 1971)

In the subjective method the subject himself has to estimate the alteration in position of a line or a cross, either by direct visual inspection or by using afterimages (Ruete, 1846 Skrebilsky, 1871, Fischer, 1927)

Consequently, we are dealing with two completely different recording methods In the objective method the investigator is studying different details in the eye, in the subjective method the subject is interpreting his own visual impressions The purpose of the present investigation has therefore been, partly to compare the two methods on one and the same person and partly to develop a quick method of investigation suitable for use in clinical practice in studying the function of the otolith organs

MATERIAL AND METHODS

Fourteen normal persons took part in the investigation They were placed with their mouths in a

which have passed into the inner ear The explanation concerning the mechanism of Arslan's surgical method, according to which the osmotic stimulation only restores the deranged biochemical and physiological balance of the inner ear, is corroborated by our results An osmotic impulse may be of importance for microcirculation within the labyrinth (Godlowsky, 1972) The same effect should theoretically be produced by compounds other than sodium chloride The question as to the causes of the severe hearing loss occurring in some patients 3–6 weeks postoperatively is nevertheless still unsolved It is safe to say that it is not an immediate effect of the concentration of sodium ions—otherwise it would be observed directly after the surgical operation Apparently the transport of sodium chloride out of the middle ear occurs very quickly, as shown by the measured values in the rinsing water Probably the main part passes via tube into the digestive tract, whence it is resorbed and excreted by the kidneys This is supported by the radioactivity found in blood and urine

Finally, a direct resorption via the mucous membranes of the middle ear may also be discussed Our time for investigation was limited the brief half life of ^{24}Na, but in long term periments no other results are to be expected, cause of the quick transport out of the middle ear

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sion between 2° and 18° was obtained The individual dispersion, however, was only 0°–3° The mean value of the counter rolling of the whole group was 9 37°, and the mean value of the dispersion 1 78°

The subjective estimations showed wide variations from subject to subject The lowest indication was 1°, and the highest 21° with a mean value of 10 04° The individual dispersion varied from 3° to 10° with a mean value of 4 71°

A comparison between the objective and the subjective values also showed wide variations Cases 10 and 11 had the same objective values (13°), whereas the subjective values showed large differences, 19 6° and 5° respectively On the other hand, cases 4 and 13 had the same subjective values 11 8°, but the objective recordings showed entirely divergent values, 6 8° and 16 6 respectively

DISCUSSION

The results of the objective recording showed † generally, and irrespective of the magnitude ˙ the recordings, the variation was 0–3° Con uently, the variation may be attributed to ıı ɹ in reading Two spots on the iris on ı sides of the pupil are usually looked for, sometimes it is impossible to find more than and one must be satisfied with that ...her with locating the centre of the pupil his, however, makes the recording somewhat ertain, because the centre of the pupil is a real point, but must be estimated from the iphery of the pupil Thus is possibly why, four cases, there was an error in reading of 3°

The estimations made with the subjective ɹ showed that the subjects had considerbly greater difficulties in estimating the apparnt horizon The error is never less than 3° nd may amount to 10°

If the objective and the subjective methods re compared it will be found that they do ot always show the same relation to the angle f tilting, even if the statistical mean values are bout the same This must be attributed to ɛ uncertainty of the subjective estimations

From the findings of the investigation it is concluded that the objective method is much more reliable and better reproducible than the subjective It must therefore be regarded as both a reliable and a quick recording method, suitable for the clinical investigation of the influence of the otolith organs on the oculomotion and of counter-rolling in particular (Fluur & Siegborn, 1974)

ZUSAMMENFASSUNG

Um eine schnelle und zuverlassige Methode fur die Registrierung von Gegenrollung als einen Indikator der Funktion der Otolithen zu entwickeln wurden 14 normale Personen sowohl mit einer objektiven als auch mit einer subjektiven Registrierungsmethode untersucht Fur die objektive Registrierung wurde ein Goniometer-Okular auf einem Zeiss Mikroskop verwandt und fur die subjektive Methode ein leuchtendes in den drei Achsen des Raumes bewegliches Kreuz Die Resultate zeigen dass die objektive Methode viel zuverlassiger und besser reproduzierbar ist als die subjektive und folglich eine gute und schnelle Registrierungsmethode fur klinische Untersuchungen der Gegenrollung darstellt

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POSTINFLAMMATORY ACQUIRED ATRESIA OF THE EXTERNAL AUDITORY CANAL

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Abstract Seventeen cases of postinflammatory acquired atresia of the external auditory canal in 14 women are described and analysed Fourteen of these cases were solid atresias in which the auditory canal was more or less filled with fibrous tissue Three patients had partial membranous atresia The aetiology was external otitis and/or chronic otitis media The formation of atresia is usually by jerks and its steps are as follows External otitis with destruction of the epithelium, formation of granulations, fibrosing of the granulations and lining with new meatal skin

Acquired atresia of the auditory canal is fairly uncommon Aetiologically it may be sub divided as follows *Post-traumatic atresia* arising as a result of severe, direct or indirect, trauma, such as shotgun injuries (Conley, 1946), severe contusions of the ear (Proud, 1956), and impression fractures of the anterior meatal wall with displacement of the mandibular neck (Anthony, 1957) It may also follow upon burns, thermal, chemical or electric (Proud, 1956, Cohen & Fox, 1943) *Postoperative* atresia arises as an early phenomenon following otosurgery, and in rare cases after surgery on the parotid gland (Proud, 1956) Furthermore, *neoplastic* atresia may occur when a malignant tumour from the auditory canal or its surroundings entirely occludes the auditory canal (Mitchell, 1940, Work, 1950) The aetiology and pathogenesis of these three varieties are evident and easy to understand As a rule these cases are preceded by stenosis, whereupon total closure takes place because of granulations The presupposition of atresia is that the skin is damaged in the entire circumference of the auditory canal Between the atresia and the drum the meatal epithelium may be preserved, and a meatal cholesteatoma results

The main object of the present communication is to describe a fourth variety of acquired atresia, *postinflammatory* acquired atresia Only a few cases of this type are on record, and its pathology, aetiology, or pathogenesis do not appear to have been entirely elucidated

PREVIOUS INVESTIGATIONS

In the literature we have found only seven cases of postinflammatory acquired atresia Novick (1939) reported membranous atresia in a man with a history of recurrent meatal complaints many years ago Work (1950) described bilateral acquired atresia following chronic otitis media

Gundersen (1960) described three cases of acquired meatal atresia In the first case the atresia was found to consist of fibrous tissue Close to the drum there was a plate of new formed bony tissue In the second case the atresia was situated laterally in the auditory canal Between it and the intact drum were large exostoses and cholesteatoma-like epithelial masses In the third case the atresia was 5 mm lateral to the drum Between the drum and the atresia there was fibrous connective tissue

Eichel & Simonton (1965) reported on a patient who developed, following external otitis, bilateral fibrous atresia 3 mm lateral to the drum

Table I *Presentation of material*

CASE NO AGE	PRIMARY EAR DISEASE	FIRST OTOSCOPY	FURTHER COURSE	HEARING A D	POSSIBLE AETIOLOGY	HEARING AIR BONE	MEATAL SKIN	PATHOLOGY OF MEATAL ATRESIA 1971 1973
1 (43)	O E OF 4 MONTHS DURATION IN 1964	1964 GRANULATIONS ON THE DRUM 1969 INC P ENT ATRES A	RECURRENT O E IN 1971 AND 1972 PROGRESS ATRESIA		O E	40/10	NORMAL	
2 (45)	O M C DUR NG SEVERAL YEARS	1960 LARGE DRY CENTRAL PERFO-RAT ON E M NORMAL	RECURRENT DISCHARGE AND O E	1960 – 1965	O M C	40/10	DRY O E	
3 (46)	O M C DUR NG SEVERAL YEARS	1960 LARGE MO ST CENTRAL PERFORA TION E M NORMAL	RECURRENT D SCHARGE AND O E	1965 972	O M C	40/ 0	DRY O E	
4 (7)	CHRON C O E	1956 AND 1964 NORMAL DRUM NORMAL E M 1966 ATTACK OF C E	RECURRENT O E IN 1967 1969 AND 1970		O E	60/10	MO ST O E	mm
5 (70)	CHRON C O E	1956 NORMAL DRUM NORMAL M E 1964 O E W TH GRANULATIONS ON THE DRUM	RECURREN O E IN 1966 1967 1969 AND 1970		O E	60/10	MO ST O E	22mm
6 (72)	RECURRENT ACUT OT TS MED A N CH LDHOOD PERCEPT VE DEAFNESS	1957 N ACT SLIGHTLY F BROUS DRUM E M NORMAL 1966 O E	RECURRENT O E	1956 1966	O E	60/45	DRY O E	2
7 (53)	O M C DUR NG SEVERAL YEARS	1966 SMALL MO ST CENTRAL PERFO-RAT ON O E	CONSTANT Y D SCHARGE		O M C	55/25	MO ST O E STENOSIS OF E M	
8 (64)	O M C SINCE CHILD-HOOD	1967 LARGE MO ST CENTRAL PERFORA T ON O E	D SCHARGE UNT L 1970 DRY EAR S NCE	1960 1973	O M C	90/30	NORMAL	
9 (50)	O M C S NCE CH LDHOOD	1962 LARGE MO ST CENTRAL PERFORA T ON	RECURRENT O E 1969 INCI P ENT ATRE S A DRY	FROM 1962	O M C	50/10	NORMAL	

OMC = Otitis media chronica OE = Otitis externa EM = External auditory meatus

The fibrous tissue contained many dilated blood vessels

Marlowe (1972) described a patient who developed bilateral atresia with meatal cholesteatoma four months after an infection in both auditory canals

MATERIAL AND RESULTS

The material consists of 14 patients with postinflammatory acquired atresia in a total of 17 ears, as in three cases the atresia was bilateral (Table I)

Incidence

During a two year period we have collected twelve cases of postinflammatory acquired atresia among 3 770 audiological patients over 14 years of age—an incidence of one atresia in about 300 patients In a retrospective review of 600 patients who had tympanoplasty, we found two patients with postinflammatory acquired atresia, corresponding to an incidence of one atresia per 300 tympanoplasties

A striking finding was that all the patients were females Among the 7 patients on record 5 were males

CASE NO AGE	PR MARY EAR D SEASE	F RST OTOSCOPY	FURTHER COURSE	HEAR NG A D	POSS BLE AETIOLOGY	HEAR NG A R BONE	MEATAL SK N	PATHOLOGY OF MEATAL ATRES A 97 1973
10 63	O M C S NCE CH LDHOOD	1957 AND 1962 LARGE MO ST CEN RAL PERFO RAT ON 1962 DRY PERFO RA ON	O E FROM 1962 1968 DRY NC P ENT A RES A	FROM 1962	O M C	90 30	NORMAL	
11 80	PER EPT VE DEAFNESS	1960 NORMAL DR M E M N RMAL	FROM 968 RECURRENT O E 969 INC P ENT ATRESIA	FROM 1968	O E	95/40	DRY O E	
12 61	O OSCLERO S S	1954 AND 965 NORMAL DRUM E M NORMAL	FROM 968 CHRON C O E	1956 1969	O E	95 40	DRY O E	
13 6	O OSCLERO S S N 948 FE NESTRAT ON OPERAT ON	1965 DRY FENE STRA ON CAV TY E M NORMAL	FROM 970 CHRONIC O E W TH O S CHARGE	FROM 1969	O E	90/55	MO ST O E	26mm 6mm
14 71	O OS LERO S S 958 FENESTRA T ON OPERA T ON	1965 DRY FENE STRAT ON CAV TY	RECURRENT O E		O E	6 /40	MO ST O E	27mm 5mm
15 30	O M C DUR NG SEVERAL YEARS PERCEPT VE DEAFNESS	1955 LARGE DRY CENTRAL PERFORAT ON	RECURRENT D S HARGE		O M C	10 /100	MO ST O E	27mm
16 46	O M C S NCE CHILDHOOD 1937 A T CO AN TROTOMY	1962 M NOR D S CHARG NG CAV TY W TH GRANULATIONS	DRY FROM 1963		O M C	6 / 0	NORMAL	16mm
17 25	O M C S NCE CH LDHOOD	963 LARGE PER FORAT ON OF THE DRUM	CON NOUS D SCHARGE DRY FROM 1965		O M C	75/35	NORMAL	28mm

Two patients were 21–40, 5 were 41–60, and 7 were 61–80 years of age The youngest was 25 and the old~st 80 (Table I), mean 56 years

PATHOLOGY

In most cas~s the atresia was situated medially, i e in the osseous part of the auditory canal, in 4 cases in the middle of the meatus, at the junction of the cartilaginous and osseous part (Table I Cases 8–10 and 16 Fig 1) and in 2 cases laterally (Cases 11 and 12) The meatus lateral to the atresia was somewhat narrow in one of the patients, who had moist eczema (Case 7) but no other patients had stenoses In several cases the lateral part was even rather wide Just before the atresia the auditory canal narrowed from all sides, ending in an epithelial lined atretic plate In 5 patients, moreover, the meatal skin lateral to the atresia was eczematous and rather moist In 5 cases there was dry, scaly external otitis (Fig 2) while in the remaining 6 the meatal skin was normal

Physical examination radiography, and operation disclosed two types of atresia (1) solid atresia and (2) membranous, web type atresia

Solid atresia

In solid atresia the auditory caral between the atretic plate and the drum may be filled either with (*a*) fibrous tissue, (*b*) fibrous tissue with cholesteatoma or (*c*) fibrous and osseous tissue

(*a*) *Solid fibrous atresia* was found in 13 cases (Table I, Cases 1–12, Case 17) Six were treated by operation (Cases 1–3, 10 12, and 17), which disclosed between the atretic plate and the drum,



Fig 1 Solid atresia after chronic otitis media Developed after treatment with hearing a d (Case 9)

fibrous tissue characterized histologically connective tissue containing numerous capillaries and non specific sub chronic or chronic inflammatory changes The fibrous tissue was adherent to the lamina propria of the drum but could easily be separated and removed from the mucous layer In cases where a perforation of the drum had been demonstrated previously (Cases 2 3 and 10) operation showed that in this site the mucous layer was intact and the perforation had healed However, an exception was found in Case 17 in which the middle ear housed a cholesteatoma arising from the margins of the perforation The osseous parts of the auditory canal and of the middle ear were normal

(*b*) *Solid fibrous atresia with meatal cholesteatoma* was observed in one case (Case 16) At operation the greater part of the atresia was occupied by fibrous tissue of the same histological architecture as described above Apart from this there was anteriorly, in the inferior part of the auditory canal close to the drum a well defined cholesteatoma The lamina mucosa and drum were intact, and there was no cholesteatoma in the tympanic cavity In 1936 this patient had undergone attico antrotomy because of chronic otitis media In 1962 there was a small cavity but no atresia of the auditory canal The cavity wall was lined with granulations which had presumably spread inferiorly and anteriorly, so that the epithelium from the inferior tympano meatal angle became surrounded by granulations

(*c*) *Solid fibrous osseous atresia* was not found in the present material but has been described by Gundersen (1960)

Membranous atresia

Membranous atresia was present in three cases (Table I, Cases 13 15) but the membrane was not quite closed Two patients had previously had otosclerosis treated by fenestration which functioned satisfactorily for many years The skin in the cavity was very thick and the cavity



Fig 2 Solid atres a with epithelial l ned granulations in the central area Dry eczema in the auditory canal (Case 3)

was almost completely obliterated Through the aperture in the atretic membrane the drum could be seen moving normally Medial to the atresia the meatal skin was eczematous and moist The third patient (Case 15) had chronic otitis media with a large central perforation of the pars tensa The auditory canal between the atresia and the drum was filled with granulations arising partly from the middle ear and partly from the auditory canal

AETIOLOGY

It was common to all 17 of the present cases that prior to the development of atresia there had been an inflammatory condition in the auditory canal and/or middle ear

Chronic or recurrent *external otitis* was in 8 cases the direct cause of the atresia (Table I, Cases 4–6, 11–14) In these cases the drum had been, when first examined several years ago, intact, and the meatus had been normal Four patients (Cases 6, 11, 12, 13) developed external otitis 6 to 12 months after they had been fitted with hearing aids It had persisted or recurred for some years, and had been localized in the main medially and close to the drum

In the remaining 9 cases the patients had primarily had *chronic otitis media* (Cases 2, 3, 7–10, 15–17) For years before the atresia developed, they had had chronic or recurrent aural discharge, and when first seen several years ago they had exhibited perforation of the drum Four patients (Cases 2, 3, 9, 10) had a normal auditory canal when first seen, but thereafter developed external otitis after they started using hearing aids A total of 6 patients had, besides the chronic otitis media, definitely had chronic external otitis for some years before the atresia appeared In the remaining 3 cases the external otitis was probably present for some part of the course

Within the entire material 9 patients had been wearing hearing aids for years before the atresia appeared In two, with bilateral atresia (Cases 2, 3, 12, 13) the external otitis and the subsequent atresia occurred first in the ear in which the hearing aid treatment was started After the hearing aid had been shifted to the other side, external otitis occurred there too, and so did atresia In patients having a tendency to external otitis, occlusion of the auditory canal by an earmould will—by reason of local irritation, increased moisture, and possibly allergy to the material in the mould—aggravate the external otitis and cause accumulation of moist debris in the innermost part of the auditory canal This forms the basis of the local changes which may lead to the formation of meatal atresia

PATHOGENESIS

In *solid fibrous atresia* the auditory canal fills, gradually or by jerks, with fibrous tissue, starting on the drum and progressing lateralward In most patients we have been able to trace the course of the disease several years back In one patient (Table I, Case 1) the formation of the atresia was regularly observed by us In 1964 this patient, who had no previous history of meatal complaints, developed severe unilateral external otitis which persisted for 4 months despite treatment On the drum there were granulations, whereas the lateral part of the meatus was only mildly involved (Fig 3 A) The granulation subsided, and the drum became re epithelized, thereafter, it was somewhat thickened, mobile, but with distinct details In 1969 the patient had another attack of external otitis and the process was repeated

At paracentesis and tubulation under the operation microscope the atresia was 1½ mm thick, immobile, and consisted of firm, fibrous tissue covering the handle of the malleus (Fig 3B) The middle ear was aerated and the middle-ear mucosa normal In 1971 an identical attack occurred, and the atresia increased in thickness by another ½–1 mm In 1972 the patient again developed marked formation of granulations at the site of the atretic plate and medially in the auditory canal The granulations started peripherally in the atretic membrane and in the meatal wall, spreading towards the middle, and the original epithelium disappeared completely Gradually, epithelium grew from the meatal skin over the granulations which decreased in size and grew firmer (Fig 4) At the operation

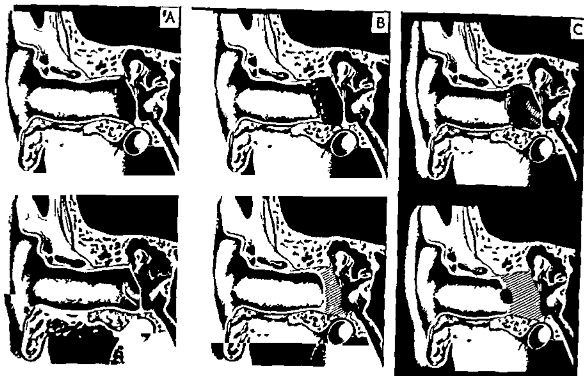


Fig 3 Schematic representation of the development of solid atresia (Case 1) (A) First attack of otitis externa with granulations on the ear-drum and the resulting thickened fibrous ear-drum after re-epithelization (1964) (B C) The subsequent attacks progressing atresia after each attack (1969 and 1972)

the entire atretic membrane, except for a small ea in the middle, was lined with meatal skin 3C)

Fig 4 Development of solid atresia Central part moist with granulations peripheral part lined with skin (Case 1)

In this case the solid fibrous atresia grew jerkily over several years and the same process went on repeating itself External otitis with denudation of the epithelium and formation of granulations medially in the auditory canal fibrosing of the granulation tissue, and lining of the granulations with meatal skin The primary factor of the process was necrosis of the meatal skin and denudation of all epithelial elements Signs of a similar course were observed in other patients in whom the aetiology was *external otitis and in whom the drum as well as* the auditory canal were originally normal They had recurrent external otitis (Cases 4–6 10–12) formation of granulations on the drum (Case 5) or incipient atresia which progressed in the course of the subsequent years (Case 11)

The observed level of the atresia can probably not be considered the final state as the atresia may spread further laterally At re-examination of the non operated cases one year later, we found that in one case the cutaneous lining of

the atresia had disappeared, being replaced by new granulations

In cases that started as chronic otitis media with perforation of the drum (Cases 2, 3, 7, 8–10) the perforation of the drum must have closed in the first stage of formation of the atresia In the operated cases (Cases 2, 3, 10) the inside of the drum proved to be lined with mucosa The subsequent development of atresia presumably takes place in the way described above After the formation of atresia had started, the discharge decreased, and the patients had thereafter had only recurrent external otitis (Case 9) or an entirely dry ear for some years (Cases 2, 3, 8–10)

The condition for non-occurrence of cholesteatoma in the auditory canal is that all the squamous epithelium from the drum or the edge of the perforation must be destroyed before it gets covered with granulations In two cases, however, operation revealed cholesteatoma In the former (Case 17) the cholesteatoma sac was situated in the tympanic cavity It had arisen from the edge of the perforation which was not lined with mucosa, so that the sac was in direct contact with the fibrous atretic tissue It is difficult to tell whether the atresia had formed after or before the invasion of the cholesteatoma sac into the tympanic cavity In the latter case (Case 16) there was, as already mentioned, a cholesteatoma anterior in the auditory canal, close to the drum which was incidentally intact The cause of cholesteatoma formation was presumably that the atresia coursed from the roof to the floor of the auditory canal and thus enclosed epithelial remnants inferiorly, unlike the solid fibrous atresia which coursed from the drum laterally

The pathogenesis of *membranous* atresia is different from that of solid atresia According to Work (1950) the formation of a membrane laterally in the auditory canal is due to filling of the auditory meatus with pus and detritus, resulting in special local irritation at a given level of the meatus There will be ulceration in the entire circumference, and granulations will form The granulations get epithelized from both

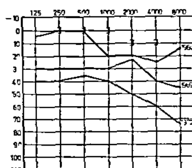


Fig 5 Progressing air bone gap during the development of solid atresia (Case 1) as illustrated in Fig 3

sides, and a membrane results The epithelium of the auditory canal and drum will be enclosed, with accumulation of epithelial masses, behind the atretic membrane

The reason why the membrane did not close completely in our cases may be that the skin in the medial part of the auditory canal was still affected with external otitis with discharge (Cases 13, 14) The third patient (Case 15) still has active chronic otitis media with formation of granulations, arising partly from the middle ear and partly from the medial part of the auditory canal (Case 15)

The pathogenetic process in the web-type atresias has presumably not yet been completed, and the membrane may close entirely On the other hand, it is conceivable that the epithelium medial to the atresia may be destroyed upon renewed formation of granulations, the granulations may undergo fibrosis, and the aperture in the atretic membrane may close Thus, the web type of atresia may become solid, fibrous atresia

HEARING

For most of the patients audiograms are available for the time before atresia formed, but the great majority had even then considerable hearing loss of the conductive type, either because of chronic otitis media with perforation or otosclerosis Some had perceptive hearing loss

In one patient a relationship between the growth of the atresia and the hearing loss could be demonstrated (Table I, Case 1) After the first

attack of granulation formation in 1964 (Fig 5), when the drum was merely rendered somewhat thicker, but remained mobile, the hearing was 15 dB After the second attack in 1969, when the drum increased in thickness to 1½ mm and became immobile, hearing decreased to 30 dB, and at the most recent attack in 1972, when the atresia had become 7 mm thick, the hearing was 40 dB

Table 1 gives the hearing in the frequency range 500–2 000 Hz at the most recent examination In 3 patients (Cases 1–3) exhibiting at operation a normal middle ear and a normal ossicular chain, the air bone gap was 30 dB, and in one patient (Case 16) it was 50 dB In a few others, not subjected to operation, in whom radiography indicated that the middle ear was normal, there was an air–bone gap of 30–60 dB (Cases 4–9) The most pronounced hearing loss occurred during incipient atresia formation Later, the hearing did not decrease essentially, even despite a manifold increase in thickness of the atresia

TREATMENT

nce the atresia has developed, the only effective ment is surgery Seven patients (Table I, 1 3, 10, 12, 16 17) underwent operation which the atresia was completely excised and the auditory canal as well as drum were covered with free skin grafts In the same stage other pathological changes in the middle ear were treated The technique and results will be published elsewhere Laterally situated atresias involve rather difficult problems in re epithelialization of the auditory canal and drum

In patients who have had medially situated atresias, early operation is preferable Removal of granulations from the drum may perhaps prevent the occurrence of the atresia As treatment of patients with external and chronic otitis by means of a hearing aid presumably promotes the formation of atresia annual follow up of such patients may disclose incipient signs of this process

DISCUSSION

The aetiological classification of acquired atresias suggested in the present paper seems justified, as the aetiology of traumatic, postoperative, and neoplastic atresias is evident It has also been demonstrated in the present study that recurrent external otitis, alone or combined with chronic otitis media, may be the cause of post inflammatory acquired atresia In our opinion therefore, this term is adequate for the disease and clearly distinguishes it from other types of atresia

Postinflammatory atresia does not appear to be as rare as previously assumed It will be found especially among elderly patients who have been wearing a hearing aid for many years or who have been fitted with a hearing aid for an ear with perforation of the drum No doubt the improved audiological service and following up of these patients is contributory to the increasing incidence of acquired atresia

Although the diagnosis of a full blown atresia is easy, incipient formation of atresia with granulations on the drum may give rise to diagnostic considerations pointing to acute and chronic otitis media and tumours

Shambaugh (1969) has described a state of chronic granulation formation on the drum chronic myringitis This condition seems to resemble the initial stage of postinflammatory acquired atresia with which it is possibly identical However, in atresia the granulations arise from the meatal skin close to the drum

Solid postinflammatory atresias are more common than the web type ones, whereas among the post traumatic cases the web type is more common than solid atresia (Conley 1946) This is understandable, as it is a precondition for the occurrence of web type atresia that a circular injury to the meatal skin occurs lateral to the drum

ZUSAMMENFASSUNG

Bei 14 Frauen wurden 17 Falle mit postinflammatorischen Gehorgangsatresien beschrieben und analysiert Bei 14 Fällen war die Atresie solide und der Gehorgang war

ganz oder teilweise mit fibrosem Gewebe ausgefüllt Bei drei Fallen war die Atresie membranos Die Ätiologie war Otitis externa und/oder Otitis media chronica Die Atresie wird in der Regel ruckweise gebildet, unter fol genden Prozessen Otitis externa mit Auflosung des Epithels Bildung und Fibrosierung des Granulations gewebes, Überdeckung des Granulationsgewebes mit neuer Gehorgangshaut

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SELECTIVE DENERVATION OF THE AUTONOMIC NERVE SUPPLY OF THE NASAL MUCOSA

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Abstract The autonomic innervation of the nasal mucosa has been studied in normal rats and in rats in which the supplying nerves had been selectively transected post operative survival times varied from 2 weeks up to 18 months The vascular structures appeared to be innervated both by acetylcholinesterase and noradrenalin containing fibres Furthermore a nerve plexus consisting of both types of fibres was observed in the subepithelial region The nasal glands however showed only a cholinergic innervation The pattern of the autonomic nerve supply deduced from these selective denervation experiments does not differ fundamentally from the generally accepted scheme of this system in the nose of other mammals Denervation occurred within 2 weeks after transection of the supplying nerves, but after longer survival times reinnervation was observed

as been suggested that vasomotor rhinitis inates from an "imbalance" of the autono- c nervous system, through a predominance of the parasympathetic innervation (Golding-Wood, 1963, Whicker, 1973) Autonomic fibres pass to the respiratory part of the nasal mucosa via the pterygopalatine ganglion Various methods directed towards inactivation of this ganglion have been attempted in the treatment of vasomotor rhinitis In the last decade transection of the vidian nerve, the main pathway via which parasympathetic fibres reach the pterygopalatine ganglion and thence the nose, has been advocated as the surgical therapy of choice for vasomotor rhinitis and even for allergic rhinitis Good results have been reported with this method (Golding-Wood, 1961, 1973 Hiranandani, 1966, Chandler, 1969 Montgomery et al, 1970, Wentges, 1973) However there is little basic physiological knowledge of the behaviour of the nasal mucosa whether under normal circumstances or after denervation

In recent years the pattern of innervation of the nasal mucous membrane has been investigated by means of histochemical and fluorescence techniques Dahlstrom & Fuxe (1965) demonstrated the presence of adrenergic nerve fibres in the wall of the nasal vessels Ishii & Toriyama (1972) and Nomura & Matsuura (1972) showed the presence of acetylcholinesterase-containing fibres around the mucosal vessels and glands Experiments in which autonomic nerves were transected have been performed by Krajina et al (1972) In four dogs they observed morphological changes in the mucosal vessels and glands in biopsy specimens taken some days after bilateral cervical sympathectomy

The present study includes both short- and long term experiments, their objective is to determine the behaviour of the autonomic nerve endings in the nasal mucosa following selective transection of the appropriate nerves

METHODS

The experiments were performed on Wistar rats (about 200 g body weight) The animals were anaesthetised with Nembutal (30 mg/kg body weight) administered intraperitoneally A diagram of the generally accepted course of the autonomic nerve supply of the nose in mammals is shown in Fig 1 The arabic figures in this diagram refer to the place where the nerves

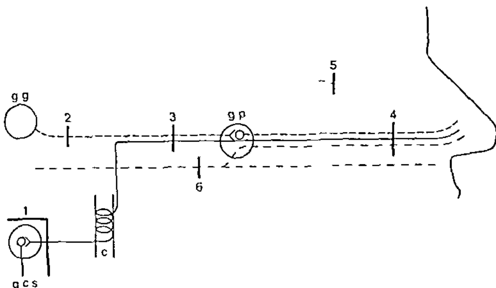


Fig 1 Diagram of the generally accepted course of the autonomic nerve supply of the nose in mammals The arabic figures refer to the place where the nerves were transected 1 Cervical ganglionectomy, 2. Transection of the greater superficial petrosal nerve 3 Transection of the vidian nerve 4 Transection of the posterior nasal nerve 5 Transection of the ethmoidal nerve 6 Transection of the maxillary nerve

Abbreviations *c* carotid artery g c s superior cervical ganglion g g geniculate ganglion g.p pterygopalatine ganglion

were transected Denervation was performed unilaterally and bilaterally The following experiments were carried out

(1) *Extirpation of the superior cervical ganglion*

This ganglion is the main source of the sympathetic innervation to the nose The superior cervical ganglion, situated near the bifurcation of the carotid artery, was reached by a ventral incision in the neck This ganglion was extirpated and the carotid plexus, which originates from this ganglion, was stripped off

(2) *Transection of the greater superficial petrosal nerve*

This nerve contains the parasympathetic nerve fibres passing to the pterygopalatine ganglion The greater superficial petrosal nerve was transected in the middle ear just beyond the geniculate ganglion The middle ear was reached by a dorsal approach

(3) *Transection of the vidian nerve*

It is concluded that this nerve contains the parasympathetic preganglionic fibres originating from the greater superficial petrosal nerve and the sympathetic postganglionic fibres from the superior cervical ganglion which form the deep petrosal nerve We were unable to identify the deep petrosal nerve in rats The vidian nerve is situated in the pterygopalatine groove Transection of the vidian nerve was carried out through the orbit A midline incision down to the bone in the orbital region was made The soft tissues were separated from the skull and then from the medial wall of the orbit Subsequently the orbital contents were retracted until the maxillary nerve was seen The vidian nerve, situated immediately inferior to the maxillary nerve, was identified and transected proximal to the pterygopalatine ganglion

(4) *Transection of the posterior nasal nerve*

The posterior nasal nerve contains postganglionic parasympathetic and sympathetic fibres as well as sensory fibres from the maxillary nerve This nerve was approached in the same way as the vidian nerve Transection was carried out just proximal to where this nerve enters the sphenopalatine foramen




Fig 2 (*a*) Whole mount specimen of the nasal mucosa showing acetylcholinesterase-containing nerve plexus in the wall of the venous sinusoids. (*b*) Section of the nasal mucosa (lateral wall) showing noradrenalin-containing nerve plexus in the wall of the venous sinusoids *L* part of the lumen of a collapsed sinusoid.

Transection of the anterior ethmoidal nerve

anterior ethmoidal nerve supplies a minor portion of the antero-superior part of the nasal mucosa This nerve was divided on the medial or bital wall at the level of the ethmoidal foramen

(6) *The maxillary nerve*

Besides sensory fibres this nerve is thought to contain sympathetic fibres (Blier, 1930 Jackson & Rooker, 1971) It is postulated that these fibres enter the pterygopalatine ganglion and reach the nose via the posterior nasal nerve The maxillary nerve was therefore divided proximal to the pterygopalatine ganglion in the pterygopalatine groove

Histochemical methods

After survival times varying from 2 weeks up to 18 months the animals were killed by an intracardiac injection of Nembutal The animals were decapitated and both the respiratory part of the nasal septum and the mucosa of the lateral walls dissected out In order to demonstrate acetylcholinesterase activity, cryostat sections (7 μm) were treated according to the method described by Karnovsky & Roots (1964) Nonspecific cholinesterase activity was inhibited by iso-OMPA (Bayliss & Todrick 1956) Noradrenaline activity was demonstrated in paraffin sections of freeze dried tissue with the fluorescence method described by Falck et al (1962) The lyophilised tissues were exposed to formaldehyde vapour and embedded in paraffin In several animals the presence of noradrenalin and acetylcholinesterase-containing fibres was studied in whole mount specimens of the nasal mucosa

RESULTS

In the normal animal both noradrenalin and acetylcholinesterase-containing fibres were ap-



Fig 3 Coronal section through the nasal septum of a normal rat showing mucosal glands with an acetyl cholinesterase-containing periacinar nerve plexus. Note the absence of nerve fibres around the secretory ducts

parent in the walls of the nasal vessels The venous sinusoids in particular showed a very dense network of both types of fibres (Fig 2) Periacinar acetylcholinesterase containing fibres were present in relation to the nasal glands (Fig 3) No adrenergic fibres could be demon strated in this region Some sections showed interconnections between the cholinergic vascu lar nerve plexus and the periacinar plexus (Fig 4) Apart from the periacinar and perivascular plexus a subepithelial plexus of both acetyl cholinesterase and noradrenalin-containing fi bres was demonstrated in the whole mount specimens

Whole mount specimens of the ethmoidal and sphenopalatine arteries showed a plexus of both noradrenalin and acetylcholinesterase-contain ing fibres

Denervation

(1) *Superior cervical ganglion and greater superficial petrosal nerve*

The results are summarized in Table I Up to 12 months after cervical ganglionectomy no adrenergic fibres could be demonstrated on the denervated sides (Fig 5) In one animal about half of the normal adrenergic activity was observed 4 weeks after unilateral ganglionectomy After longer survival times (9–12 months) re-innervation was apparent, however, in one of the bilaterally denervated animals, noradrenalin-containing fibres were absent on one side after 12 months No change in the acetylcholinesterase activity relative to normal could be observed after combined transection of the superior cervical ganglion and the greater superficial petrosal nerve (Table I) or after transection of the greater



Fig 4 Mucosal section demonstrating the connection between the acetylcholinesterase-containing vascular nerve plexus (*V*) and periacinar plexus (*A*) *L* lumen of venous sinusoid

Table I *Effect of cervical ganglion extirpation on the nerve fibres in the nasal mucosa*

Side of extirpation	Survival time	Ach esterase-containing fibres		Noradrenalin containing fibres	
		Left	Right	Left	Right
Right	2 weeks	+	+	+	−
Right	4 weeks	+	+	+	±
Both sides	7 weeks	+	+	−	−
Both sides	2 months			−	−
Right	2 months			+	−
Right	4 months[a]	+	+	+	−
Right	5 months[a]			+	−
Right	9 months[a]	+	+	+	±
Right	9 months[a]	+	+	+	+
Right	12 months	+	+	+	+
Both sides	12 months	+	+	+	−

[a] In these animals the greater superficial petrosal nerve was also transected

superficial petrosal nerve alone studied in 5 animals after survival times varying from 2 weeks up to 1 year

(2) *The vidian nerve*

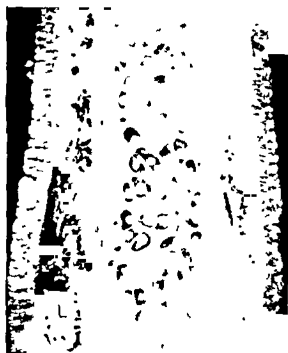
The results of vidian nerve transection are shown in Table II Adrenergic fibres were absent 2 weeks after transection of the vidian nerve Half of the normal adrenergic activity was observed in the respiratory part of the mucosa in the animals which survived for $3^1/_2$ weeks and 2 months After longer survival times a restoration to normal levels of activity was found No change in the number of postganglionic acetylcholinesterase-containing fibres was observed during the observation period

(3) *The posterior nasal nerve*

The results of the posterior nasal nerve transection are summarized in Table III Both acetylcholinesterase- and noradrenalin-containing fibres were absent up to 3 weeks after transection (Figs 6 and 7) After 2 months reinnervation seemed to have occurred (Fig 8)

(4) *Anterior ethmoidal nerve and maxillary nerve*

For both types of transection 7 animals were used After survival times varying from 2 weeks



... eeks after ... he ... m
in the walls of the vessels in the denervated side *L* vascular lumen

up to 12 months no change in the number of acetylcholinesterase- and noradrenalin containing fibres could be observed

It should be noted that some of the mucosal specimens (indicated as negative in the tables) appeared to contain small remnants of post

Table II *Effect of vidian nerve transection*

Side of transection	Survival time	Ach esterase-containing fibres		Noradrenalin-containing fibres	
		Left	Right	Left	Right
Right	2 weeks	+	+	+	−
Right	$3^1/_2$ weeks	+	+	+	±
Right	2 months			+	±
Right	8 months			+	+
Right	16 months	+	+	+	+



Fig 6 Coronal section of the nasal septum 2 weeks after unilateral posterior nasal nerve transection On the denervated side, the acetylcholinesterase-containing perivascular and periacinar nerve plexus is absent *L* vascular lumen

Table III *Effect of posterior nasal nerve transection*

Side of transection	Survival time	Ach esterase containing fibres		Noradrenalin containing fibres	
		Left	Right	Left	Right
Right	2 weeks	−	−	±	−
Right	2 weeks	−		+	−
Right	2 weeks	−		−	−
Both sides	2 weeks		−		
Both sides	2 weeks		−		
Both sides	2 weeks		−		
Both sides	2 weeks		−		
Right	3 weeks	−	−	−	−
Right	3 weeks	−		−	−
Right	3 weeks	−			
Both sides	2 months	+	+	±	±
Right	4 months	+	+	+	±
Right	4 months		−	+	±
Both sides	14 months	+	−	−	+
Both sides	14 months			+	
Right	11 months	+	−	−	
Right	18 months	−	+		
Right	18 months	−	−		

tively reacting nerve fibre fragments Because the previously noted nerve plexuses in the wall of the supplying arteries might be responsible for this phenomenon, a new series of experiments was performed In these experiments not only the posterior nasal nerve and ethmoidal nerve were transected but also the sphenopalatine and ethmoidal arteries No observable change in the residual nerve activity could be found, however

DISCUSSION

The results presented clearly demonstrate the existence of a well-developed autonomic nervous system in the nasal mucosa of the rat The vascular structures and subepithelial region appeared to be innervated by both acetylcholinesterase- and noradrenalin-containing fibres The nasal glands, however, appeared to be innervated only by acetylcholinesterase-containing fibres These

results agree with the findings of Dahlstrom & Fuxe (1965) Ishii & Toriyama (1972) and Nomura & Matsuura (1972) The vascular structures especially the venous (sinusoids) in the erectile tissue of the mucosa showed a very dense network of autonomic fibres This may be related to the regulatory function of these structures on the patency of the nasal airway (Dahlstrom & Fuxe, 1965)

The denervation experiments confirm that the superior cervical ganglion is the main source of the sympathetic nerve supply of the nasal mucosa The posterior nasal nerve constitutes the main pathway through which both post ganglionic sympathetic and parasympathetic fibres course to the nose The observed decrease of adrenergic activity after transection of the vidian nerve supports the assumption that this nerve contains postganglionic sympathetic fibres (Christensen 1934 Malcomson 1959) Apart from this the lack of an observable change in the number of cholinergic fibres in the nasal mucosa after transection of the vidian nerve and the greater superficial petrosal nerve provides confirmation of the presynaptic nature of these fibres (Tschallussow 1913 Malcomson 1959) No measurable contribution from the maxillary nerves to the autonomic innervation of the nose as suggested by Blier (1930) and Jackson & Rooker (1971) could be established with the techniques utilised here The same applies to the ethmoidal nerve Dissecting procedures revealed that this nerve in the rat only supplies the ut most antero-dorsal part of the nose

Although we were unable to demonstrate a clear contribution from the nerve plexuses of

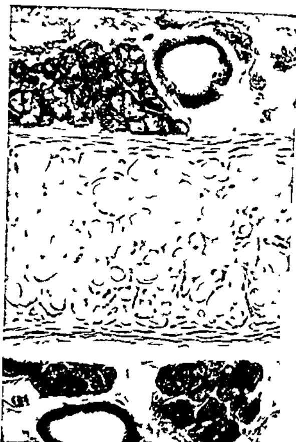


Fig 8 Coronal section of the nasal septum 2 months after bilateral posterior nasal nerve transection Restoration of the acetylcholinesterase-containing periacinal and perivascular nerve plexus has occurred

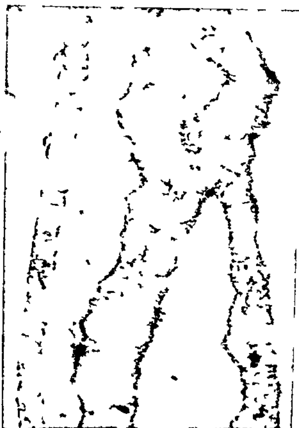


Fig 7 Whole specimen of the nasal mucosa 14 weeks after posterior nasal nerve transection The acetylcholinesterase-containing nerve plexus has disappeared from the wall of the venous sinusoids

the supplying arteries to the innervation of the nasal mucosa, such a contribution cannot be excluded However, this must be a minor one in comparison to that of the posterior nasal nerve This can be concluded from the fact that no measurable difference in residual nerve activity could be demonstrated following transection of the ethmoidal and posterior nasal nerves (*a*) alone and (*b*) after combined transection of these nerves and the supplying arteries This failure to demonstrate a significant contribution of the vascular plexus is presumably due to the choice of the survival times and/or the limitations of the techniques utilised

The small amount of residual nerve activity observed after denervation, in those experiments in which the arterial plexuses were also transected is not clear Because we were unable to establish exactly the time course of denervation and reinnervation, this phenomenon may represent either residual nerve activity after denervation, or evidence of reinnervation Furthermore, a contribution from the descending palatine nerve to the innervation of the nasal mucosa might be at least in part responsible for these observations

To summarise, these findings in the rat do not differ fundamentally from the generally accepted scheme of the autonomic nerve supply of the nose in other mammals and human beings (Malcomson, 1959 Ishii & Toriyama, 1972, Nomura & Matsuura, 1972)

The long term experiments showed a reinnervation of both sympathetic and parasympathetic systems Although we were unable to trace the actual mode of reinnervation, it seems likely that severed post ganglionic nerve fibres will regenerate from the proximal stump The appearance of new sympathetic fibres after extirpation of the superior cervical ganglion is less easy to explain After unilateral ganglion extirpation the nerve fibres from one side might cross to the other, but reinnervation after bilateral ganglionectomy must involve other sources

Apart from these histochemical observations preliminary physiological experiments showed that this reinnervation involves in many cases a restoration of the normal function of the nasal mucosa

In conclusion these studies in the rat highlight the importance of obtaining more insight into the events occurring in man after vidian neurectomy operations, particularly in the long term

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ZUSAMMENFASSUNG

Der Ursprung und die Verteilung der autonomen Nervenfasern in der Nasenschleimhaut wurde untersucht in Ratten bevor und nach selektiver Durchschneidung der zufuhrenden autonomen Nervenbahnen. Die Nasenschleimhaut zeigte eine cholinergische und adrenergische Gefässversorgung In den Nasendrusen werden nur cholinergische Nervenfasern beobachtet Ausserdem wurde ein subepitheliales Retikulum bestehend aus adrenergischen und cholinergischen Fasern dargestellt Die Befunde an normalen und denervierten Ratten entsprechen in groben Umrissen den Auffassungen uber die Nervenversorgung

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THE INFLUENCE OF VARYING AIR HUMIDITY ON MUCOCILIARY ACTIVITY

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Abstract The influence of varying humidity levels in the air surrounding the mucous membrane of rabbit tracheae has been investigated *in vitro* at 34°C, 37°C and 40 C. The following results were obtained (1) The average mucociliary wave frequency was linearly reduced following decreasing levels of relative humidity (r h.) from 90% to 20% (2) A temperature of 40 C was more detrimental to the mucociliary function than that of 34 C and 37°C (body temperature) at decreasing levels of r h (3) At decreasing levels of r h the duration of experiments with preservation in recordable activity ("mucociliary survival time") was significantly shorter (p 0 05) between 50% and 40% r h. than between 60% and 50% r h

The mucociliary activity of the mucous membrane in the mammalian respiratory tract may be influenced by many different factors such as temperature (von Gebhardt, 1909, Proetz, 1934*a*, Dalhamn, 1956, 1960, Hill, 1957, Iravani, 1967, Tanaka, 1967), relative humidity (Florey et al, 1932, Proetz, 1933, 1934*b*, 1944, Cralley, 1942*a*, Dalhamn, 1956, Toremalm, 1961, Ballenger & Orr, 1963, Ewert, 1965), mechanical trauma (Umeda, 1929, Proetz, 1933, Hill, 1957 Iravani, 1967), inhaled gases and particles (Cralley, 1942*b*, Dalhamn, 1956, Dalhamn & Rhodin, 1956 Kensler & Battista, 1966) air ions (Krueger & Smith, 1957, 1958, Andersen, 1971) and ionizing irradiation (Fujiwara et al, 1972)

However, under normal physiological conditions the temperature and humidity of the inspired air are especially responsible for the maintenance of mucociliary activity (Proetz, 1953, Rivera, 1962) The influence of the temperature parameter in the range 20 C–40°C on the rabbit trachea under *in vitro* conditions and at a relative humidity above 90% has been described in a recent publication (Mercke et al, 1974*b*)

The importance of an adequate degree of humidity for the maintenance of mucociliary activity has been pointed out in many publications especially those dealing with anaesthesiological problems (Friedman, 1955, Burton, 1962, Graff & Benson, 1969, Chamney, 1969) Specific data have only occasionally been published regarding the lower limit for affecting mucociliary activity (Dalhamn, 1956, Ewert, 1965) A systematic investigation on mammals regarding the influence of varying humidity on the mucociliary wave frequency at different temperature levels has not been found in the literature Such an investigation under *in vitro* conditions is of basic interest for further experimental research in this field

The aim of the present investigation has therefore been to record and evaluate the mucociliary wave frequency of the respiratory tract *in vitro* in rabbits during exposure to varying humidity of the surrounding air The following questions are considered

1 How does a reduction of relative humidity (r h) influence the mucociliary activity at body temperature (37°C)±3°C?

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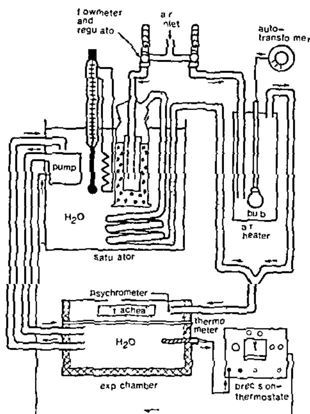


Fig 1 The experimental chamber provided with a special arrangement for regulating the temperature and relative humidity of the air flow around the tracheal speci

For how long time is it possible to work experimentally on tracheal specimens with retained mucociliary activity at different levels of reduced relative humidity at the above mentioned temperatures?

METHOD AND MATERIAL

The experimental apparatus consists of a cold light source, a binocular microscope, a photo multiplier with amplifier, a frequency filter and an ink-writer, which have been described in detail previously (Mercke et al, 1974*a*)

The experimental chamber described by Håkansson & Toremalm (1965) has been redesigned to make possible regulation and registration of temperature and r h (Fig 1) An air stream is led to a saturator where it is heated to the desired temperature and moistened by bubbling through Ringer solution Another air stream is led to a heater which reduces its humidity and raises its temperature to the same as that in the saturator Both air streams are then mixed and fed into that part of the experimental chamber where the tracheal specimen is mounted A thermocouple and a psychrometer (ELLAB type KC 1) are placed close to the tracheal mucous membrane to record the temperature and the humidity in the chamber By mixing the air streams any desired humidity can be obtained in the experimental chamber

With the exception of the space where the tracheal specimen is mounted the experimental chamber contains circulating water which is kept at a constant temperature with the aid of a precision thermostate The air from the saturator and the heater has the same temperature as the water supply of the experimental chamber

Rabbits were used as experimental animals They were killed by a blow on the head The tracheae were prepared rapidly and suspended between two perspex tubes in the experimental chamber The mucous membrane was observed from above through a hole in the upwards directed *pars membranacea*

Two experimental series have been carried out

1 Starting from 90 %, the r h has been lowered 10 % at a time down to 20 % The specimens have been exposed to each level for 30 min during which two recordings each at 30-60 sec were made The experiments have been performed at the following constant temperature levels 34°C (4 rabbits), 37°C (6 rabbits) and 40°C (7 rabbits) The duration has been 240 min

2 The maximum exposure time or 'mucociliary survival time' has been estimated at constant r h and temperatures At each of the three temperature levels, the humidity levels 60 % (5, 7 and 9 rabbits respectively) 50 % (4, 4 and 4 rabbits respectively) and 40 % (4 4 and 4 rabbits respectively) have been investigated The duration of the experiments has been limited to 120 min

RESULTS

Rabbit tracheae exposed to decreasing relative humidity levels

In Figs 2 3 and 4 the recorded mucociliary wave frequency (Table I) has been plotted

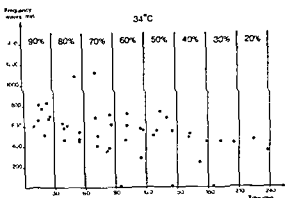


Fig 2 Diagram showing the mucociliary wave frequency at different degrees of relative humidity (%) during a period of 4 hours Constant temperature 34°C

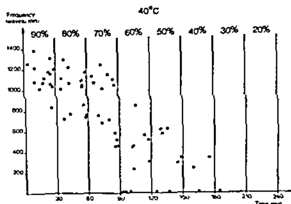


Fig 4 Diagram showing the mucociliary wave frequency at different degrees of relative humidity (%) during a period of 4 hours Constant temperature 40 C

against the successively decreasing humidity levels during 240 min and the time of recording Firstly, it appears that the initial value of the wave frequency increases with increasing temperature, from 693±56 waves/min at 34°C to 1 138±31 waves/min at 40°C Secondly, there is a tendency for the frequency to decrease following decreasing r h At 34°C the initial value of the wave frequency at 90% r h (693±56 waves/min) is reduced by approximately one-third at 30% r h (440 waves/min) This tendency to frequency reduction is also found at 37°C, and is even more accentuated at 40°C, where no specimen shows activity at 30% r h At all three temperature levels most cases of ceased wave movement can be found in the range between 60% and 40% r h

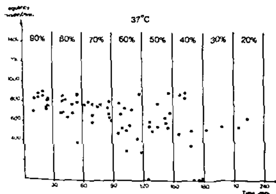


Fig 3 Diagram showing the mucociliary wave frequency at different degrees of relative humidity (%) during a period of 4 hours Constant temperature 37°C.

Fig 5 illustrates the original records from one rabbit trachea at 37°C and 60%, 50% and 40% r h These records are made with an interval of 30 min and show how the successive decrease of the r h finally is followed by ciliestasis

The average frequency at each humidity level is plotted against the respective humidity at Fig 6A–C The inclination coefficients (k) of the regression lines are 3 7 at 34°C, 3 1 at 37°C and 18 0 at 40°C (Fig 6D) The smallest inclination coefficient belongs to the regression line for 37°C, i e the body temperature of the rabbit

Rabbit tracheae exposed to constant humidity levels

In this experimental series the tracheal specimens have been exposed to constant humidity

Table I

	Mean frequency (waves/min) at		
r h (%)	34°C	37°C	40 C
90	693	807	1 138
80	602	732	991
70	597	717	850
60	534	575	445
50	625	633	509
40	424	693	288
30	440	534	—
20	409	583	—

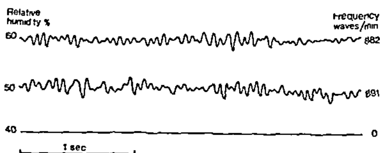


Fig 5 Typical records from one of the experiments in Fig 3 Mucociliary wave frequency and pattern at 60 %, 50 % and 40 % relative humidity Temperature 37 C. The 60 % level was reached 90 *minutes* from start 30 minutes between each record

levels at 60 %, 50 % and 40 % and three temperature levels during a maximum of 120 min The "mucociliary survival time" has been noted (Fig 7) The shorter survival at lower humidity levels is clearly shown at all three temperature levels investigated It can also be seen that the differences in "mucociliary survival time" for all three temperature levels are greater between 50 % and 40 % r h than between 60 % and 50 % r h In the former case this difference is found to be statistically significant ($p < 0.05$) according to the Wilcoxon rank sum test

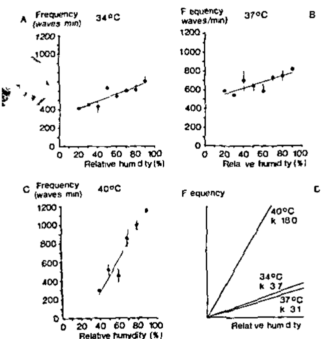


Fig 6 (A C) Regression lines calculated for the mean wave frequency of all vital tracheal specimens at different levels of relative humidity and constant temperatures (34°C 37 C and 40 C) Vertical range bars show standard error of the mean (D) The regression lines from A–C collected in one diagram showing the *k* values at the different temperatures

DISCUSSION

The mucociliary function of the respiratory epithelium is related to the body temperature *as well as to the temperature and relative humidity* of the inspired air The effect of these parameters is due to the aerodynamic conditions of the upper respiratory tract, which in turn are regulated by the anatomical configuration This relationship concerning nasal conditions has been thoroughly studied by Proetz (1953) The air conditioning ability of the larynx and trachea has been studied experimentally in models by Ingelstedt & Toremalm (1960, 1961) *The effect on the mucociliary function in the* lower part of the trachea following tracheotomy has also been investigated on tracheal specimens placed in a tracheal model during to and fro ventilation with alternating saturated air and ordinary room air to simulate expiration and *inspiration respectively (Toremalm 1961)* It was found that cilia being brought to a standstill following exposure to dry ordinary room

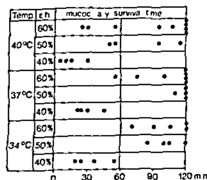


Fig 7 Diagram showing the duration of retained mucociliary activity of tracheal specimens exposed to constant levels of relative humidity (60 %, 50 % and 40 %) and constant temperatures (34°C 37°C and 40 C)

air (35–40% r h) could be resuscitated by a heat-and-moisture exchanger within a few minutes The present investigation was intended to analyse the relationship in *in vitro* experiments between mucociliary activity on the one hand and temperature, relative humidity and exposure time on the other

According to Lucas & Douglas (1934) the secretion covering respiratory mucous membrane consists of two layers The one close to the cells is runny and about 5 μm thick (Dalhamn & Rhodin, 1956) while the outer one is about 1–2 μm thick (Kilburn, 1967) and more viscous The latter is considered to work according to the sol-gel principle If the mucous membrane is exposed to an unsaturated air-flow an increased water supply is needed in order to maintain the mucociliary activity Normally, the water supply for maintaining a suitable tenacity in the mucus comes from mucus-producing cells and by transudation In *in vitro* experiments this facility is reduced of course, but the goblet cells are still capable of delivering their content for some time Thus a water loss due to evaporation into the unsaturated air stream will be compensated for during a time interval which is proportional to the relative humidity and the volume flow of the air stream The viscous mucous layer is necessary for transportation of particles but the runny layer close to the cells is enough to allow mucociliary wave movements (Sadé et al, 1970)

For comparative experimental work where mucociliary function is used as an indicator it is necessary to test the following three variable factors temperature, humidity and exposure time The influence of varying temperature at a constant humidity level of 90–100% has been investigated separately (Mercke et al, 1974*b*, Mercke, 1974) In the present paper the relation between mucociliary function and different levels of relative humidity on the one hand and the dependence of mucociliary function and exposure time on the other have been tested at and around body tempe[illegible] The effect of unsaturated air [illegible] membranes has previo[illegible] Proetz (1934*b*), Dalhamn (1956), Ewert (1965) and Tanaka (1967) However, these investigations have been performed with different methods on different animals and parts from the respiratory tract and only at one temperature level at a time The results can therefore not be compared with each other

A relative humidity of 100% in the inspired air ought to be the upper "ideal" limit for mucociliary activity as neither evaporation nor condensation of water vapour takes place But what is the gradual effect on the mucociliary activity at decreasing r h levels and how long can mucociliary activity be recorded ("mucociliary survival time") on mucous membrane specimens at lower levels of r h in the passing air stream?

The first question is illustrated by the diagrams at Figs 2, 3, 4, 6 and Table I The mucociliary frequency values are based on recordings of the type seen at Fig 5 It is obvious that the mucociliary activity is more sensitive to drying at higher temperatures This fact speaks in favour of saturated *cold air therapy* in certain respiratory tract diseases and for artificial ventilation in combination with antipyretic therapy Further conclusions for clinical purposes are naturally not possible from *in vitro* experiments only For applied and comparative studies, however, it seems necessary to control the relative humidity and keep it at a maximum or at least above 60–65% r h, which seems to be above the critical level for the preservation of mucociliary activity Moreover, the temperature should be as near as possible to the body temperature This can be deduced from the regression lines and *k* values seen in Fig 6

The answer of the second question regarding the maximum exposure time for preservation of recordable activity ("mucociliary survival time") for experimental purposes is illustrated in Fig 7 At body temperature an experimental duration of one hour is well tolerated if the r h of a permanent air stream—not exceeding 1–2 litres/min—does not fall below 50% At 40%, however, the specimens rapidly lose their capacity to produce recordable mucociliary waves This

shortening of the "mucociliary survival time" between 50% and 40% r h is statistically significant ($p < 0.05$)

ACKNOWLEDGEMENT

I am greatly indebted to Bo Dahlerus, M Sc, and Daniel Huberman, B Sc, for valuable technical and laboratory assistance

ZUSAMMENFASSUNG

Reaktionen der Trachealschleimhaut von Kaninchen auf Schwankungen der *relativen Luftfeuchtigkeit sind in vitro* bei Temperaturen von 34°, 37° und 40°C qualitativ untersucht worden

Es ergaben sich folgende Resultate

1 Bei Senkung der relativen Feuchtigkeit von 90 auf 20% erfolgte eine lineare Verminderung der durchschnittlichen mucoziliaren Wellenfrequenz

2 Die Herabsetzung der relativen Luftfeuchtigkeit *hatte bei 40°C schadlicheren Einfluss auf die muco*ziliare Funktion als bei 34°C oder 37°C

3 Die Zeitspanne des Bestehens einer registrierbaren Aktivitat („mucoziliaren Überlebenszeit") war bei rela*tiver Feuchtigkeit von 50% bis 40% wesentlich kürzer* ($p < 0.05$) als bei Feuchtigkeitswerten von 60% bis 50%

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Reaktionen der Trachealschleimhaut von Kaninchen auf Schwankungen der relativen Luftfeuchtigkeit sind *in vitro* bei Temperaturen von 34, 37 und 40 C qualitativ untersucht worden.

Es ergaben sich folgende Resultate

1 Bei Senkung der relativen Feuchtigkeit von 90 auf 20 % erfolgte eine lineare Verminderung der durchschnittlichen mucociliaren Wellenfrequenz.

2\. Die Herabsetzung der relativen Luftfeuchtigkeit hatte bei 40 C schädlicheren Einfluss auf die mucociliare Funktion als bei 34 C oder 37 C.

3 Die Zeitspanne des Bestehens einer registrierbaren Aktivität („mucociliaren Überlebenszeit") war bei relativer Feuchtigkeit von 50 % bis 40 % wesentlich kürzer ($p < 0.05$) als bei Feuchtigkeitswerten von 60 % bis 50 %.

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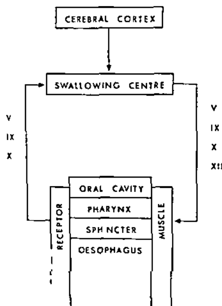


Fig 1 Diagram simplifying the neural organization of swallowing. Roman numerals refer to cranial nerves.

(Elema-Schonander) multichannel fluid jet recorder. The speed of its recording paper was 10 mm per second. On the supine test person, the nasally inserted catheter assembly was placed with the middle opening at the level of the maximum resting pressure of the pharyngo-oesophageal sphincter. Thus the pressures of the pharynx, sphincter and oesophagus were recorded simultaneously.

Test procedure

The subject was thoroughly instructed concerning the procedure of the repeated dry swallowing test. During the test he was urged to swallow 11 times, as fast as possibly, when no fluid was given. The whole test procedure was recorded.

The time between the pharyngeal pressure peaks of the first and eleventh swallowing is called the *swallowing test time*. The corresponding time between two consecutive swallowings is called *the swallowing interval*. *The relative swallowing intervals* of one test are the proportions in percentage of the swallowing intervals to the swallowing test time.

Anaesthesia

The soft palate, tonsillar pillars, tonsils, base of the tongue and pharynx (Fig 2) were intermittently sprayed with lidocaine (10% Xylocaine Dental Spray[3]) for 10 minutes, until the retching reflex was depressed. 12–18 sprayings were given. The repeated dry swallowing test was performed before and immediately after surface anaesthesia. Thus, each test person served as his own comparison.

Control

A sham procedure was performed by testing volunteers twice with a 10-minute pause, without anaesthesia.

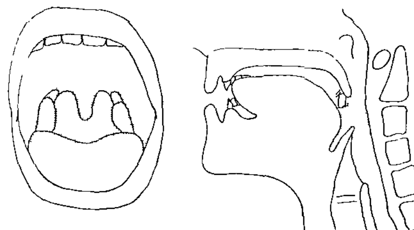


Fig 2 Anaesthetized area





Fig 3 Pressure recording of the repeated dry swallowing test before and after anaesthesia in a 50-year-old healthy female The swallowing intervals successively increased in both tests After anaesthesia the swallowing test time increased

Statistics

Only non parametric tests were used Trends were assessed by Pitman's test for correlation Contingency table analysis was also performed

RESULTS

otal material and control group

repeated dry swallowings at maximum fre uency the mean of the relative swallowing intervals of the total material successively increased (Table I Fig 3) The trend is significant ($p < 0\ 01$) The mean of the last swallowing intervals was more than twice as long as for the first ones The duration of the swallowing complexes was essentially unchanged throughout the test, the increase in relative swallowing intervals was due to increase in time between the swallows Deglutition difficulties as experienced by most volunteers appeared even after 4-5 swallowings and were in all cases obvious at the end of the test procedure Most volunteers had the impression that their deglutition difficulties at the test were due to lack of material to swallow

Inter individual variation in swallowing test time was pronounced (Fig 4) Intra individual variation was however moderate, as shown by the control group (Fig 5) Therefore each individual was used as his own comparison in the anaesthesia group Increasing age was found to be one factor correlated to increasing swallowing test time (Fig 4) The trend is significant ($p < 0\ 01$)

Table I *Relative swallowing intervals of the total material (per cent of the swallowing test time)*

	Ordinal number of swallowing interval									
	1	2	3	4	5	6	7	8	9	10
Mean	6 0	7 4	8 1	9 6	10 7	11 3	11 6	11 2	11 9	12 3
Stand dev	2 3	3 0	2 3	2 6	2 7	2 9	3 7	3 1	3 1	3 6

Anaesthesia group

After surface anaesthesia of the mouth and throat, the swallowing test time increased for 12 of the 14 subjects (Figs 3 and 6) The average increase of the anaesthesia group was 67 %

The effect of anaesthesia on the swallowing test time is statistically established by comparison between the anaesthesia and control groups (Table II) The increase in swallowing test time of the anaesthesia group was relatively evenly distributed amongst the swallowing intervals The duration of the swallowing complexes was essentially unchanged after anaesthesia The

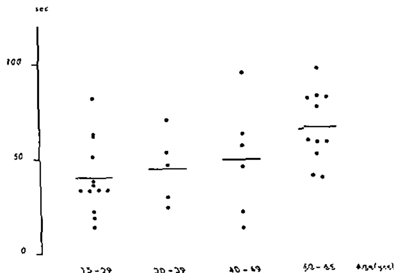


Fig 4 Total material Swallowing test times and age of the 35 subjects Each dot represents the test time of one subject, means are marked.

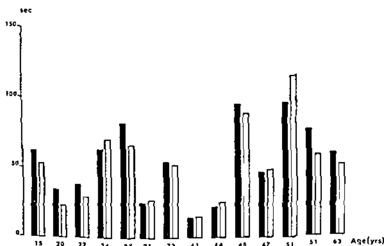


Fig 5 Control group Each subject's *swallowing test time before* (black column) and after (white column) sham procedure, 14 subjects

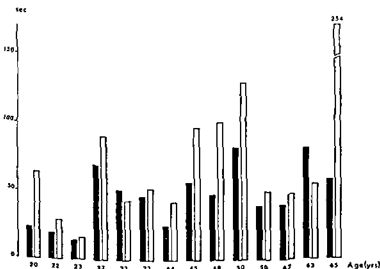


Fig 6 Anaesthesia group Each subject's swallowing test time before (black column) and after (white column) anaesthesia, 14 subjects

Table II *Change in swallowing test time after anaesthesia or sham procedure*

Each subject was his own comparison

	Number of tests with	
	Increase	Decrease
Anaesthesia group	12	2
Control group	6	8
		$p<0.05$

subjective swallowing difficulties at repeated dry swallowings increased after anaesthesia but they were of the same quality as before

DISCUSSION

The test procedures with repeated dry swallowings proved useful for studies on the swallowing reflex near the threshold level If externally supplied fluid had been used as a stimulus, the saliva would have been a disturbing factor The manometric technique used provided an objective recording of the swallowing response

Water and saliva are adequate stimuli for the swallowing reflex (Miller & Sherrington, 1915, Storey, 1968*a*) The speed with which the reflex can be repeated is dependent on the speed with which the stimulus is supplied (Månsson & Sandberg, 1974*b*) The increasing swallowing difficulties during the repeated swallowing test without anaesthesia might be due to diminishing saliva stimulus A contributary cause of the increasing difficulties in repeated dry swallowing with increasing age, might be diminished saliva secretion by age (Bertram, 1967)

Oro-pharyngeal surface anaesthesia in animal experiments was found to diminish the response of the afferent nerves of the throat on mucosal stimulation (Sumi, 1964, Storey, 1968*b* Car, 1970) In the present investigation, the surface anaesthesia seemed to cause an elevation of the threshold level of the swallowing reflex whereby increased stimulus was needed for the elicitation of the reflex Consequently the swallowing test time increased after anaesthesia No report has been found in the literature showing that oro-pharyngeal surface anaesthesia would affect secretion of saliva. Such an effect of importa[nce] seems improbable under the present experimen[tal] conditions with surface anaesthesia of limi[ted] areas and resting secretion of saliva Stud[ies] elucidating the effect of mucosal anaesthesia [on] the saliva secretion are, however, of interest.

In anaesthetized sheep, Car (1970) found [on] stimulation of the cerebral cortex that swallo[w]ing patterns were recorded in spite of the fa[ct] that reflexive eliciting was prevented by severin[g] the afferent nerves of the reflex or by curarizi[ng] the animal According to the present investiga[]tion, however, cerebro-cortical elicitation [of] swallowing in man seems to be able to instant[ly] and fully replace the reflexive elicitation sin[ce] swallowing difficulties arose when the cond[i]tions for the reflex deteriorated through dimi[n]ishing stimulus and desensitized receptors.

The findings in the present investigation gi[ve] further evidence of the importance of intac[t] oro-pharyngeal sensitivity for the elicitation o[f] swallowing According to earlier studies, sensi[]tivity is also important for the coordination of th[e] pharynx and pharyngo-oesophageal sphincter i[n] swallowing (Månsson & Sandberg, 1974*a*) an[d] presumably for the coordination of deglutition and respiration (Ogura et al, 1964) The sensitivity of oro-pharynx should therefore be considered in surgery

ZUSAMMENFASSUNG

Bei wiederholtem Trockenschlucken mit schnell[er] Frequenz, zeigte die intraluminale Druckmessung bei ge[]sunden Testpersonen eine Zunahme der Schluckinter[]valle Nach Oberflachenanästhesie des Oropharynx war das Trockenschlucken schwieriger Dies war bei einer Kontrollgruppe ohne Oberflachenanästhesie nicht nachweisbar Die Ergebnisse zeigen, dass die Auslösung des Schluckreflexes von der Sensibilität des Oropharynx abhängig ist und dass die reflektorische Auslösung des Schluckens nicht unmittelbar und vollständig von anderen Auslösungsmechanismen ersetzt werden kann.

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SQUAMOUS CELL CARCINOMA OF THE GINGIVA

Histological Classification and Grading of Malignancy

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Abstract A histological classification and grading of malignancy has been performed on the initial biopsies from 124 patients with squamous-cell carcinoma of the gingiva treated during the period 1958–69 at the Karolinska hospital Six different morphological parameters were evaluated with respect to the tumour cell population and the tumour-host relationship estimated in terms of a four point scale This permitted grading with total points ranging from 6–24 A strong correlation was found between the degree of histological malignancy and the fatal outcome of the disease Thus a tumour with a point value of 16 almost always led to the death of the patient while the best results were achieved with surgery alone i the group of small cancers (T1 + T2) with low total , points

; ie biological activity of squamous cell carcinomas of the gingivas has usually been evaluated by classifying the tumours as highly, moderately, or poorly differentiated This classification has in the past been applied despite the rather heterogeneous morphology of squamous cell carcinomas Thus, the tumours are frequently highly differentiated at the surface and less differentiated in the invasive, deeper portions Furthermore, precise definitions of the degree of differentiation of individual tumours are lacking The correlation between different degrees of differentiation of squamous cell carcinomas and prognosis has proved of limited significance and this type of classification is therefore in many cases insufficient as the basis for the choice of therapy

It is therefore imperative to try to find more objective criteria for the histological classification of gingival carcinomas which better reflect the biological growth capacities of the tum and might therefore serve as an improved to the therapy and prognosis in individual ca

At Radiumhemmet and the Department Tumour Pathology an attempt has recently b made to classify squamous cell carcinomas the larynx according to new principles (Jak son, 1973, Jakobsson et al, 1973) A sim modified classification has been applied to sq mous cell carcinomas of the palate (Eneroth Moberger, 1973) and to squamous cell carci mas of the gingiva (Willen & Nathanson 197

MATERIAL

The material consists of 124 patients with squ mous cell carcinoma of the gingiva treated Radiumhemmet and the Department of Ot laryngology, Karolinska sjukhuset, Stockho during 1958–69 The cases constitute 35% of such gingival carcinomas reported in Swed during the same period Of the patients, 80 we men, and 44 women, aged between 40 and years (mean, 69 years) 76% of all patients f within the age range of 50 to 80 years The p mary tumour was located in the lower gum 82 patients and in the upper gum in 42 cases

METHODS

The histological reclassification has in all cas been performed on the initial biopsies, prior

Table I *Histological grading of malignancy*

	I Tumour cell population			
	1	2	3	4
Differentiation	Highly, keratinization	Moderately, some keratinization	Poorly minimal keratinization	Poorly no keratinization
Nuclear polymorphism	Few enlarg nuclei	Moderate number of enlarged nuclei	Numerous irregular enlarged nuclei	Anaplastic immature enlarged nuclei
Mitoses	Single	Moderate number	Great number	Numerous

treatment The morphological analysis was made without any knowledge of the clinical stage, the treatment, or the further course of the disease The tumour cell population and the tumour–host relationship were considered separately The evaluation of the *tumour cell population* was based upon a grading of the degree of differentiation, the nuclear polymorphism, and the frequency of mitotic figures, in terms of a 1–4 point scale (Table I) The *tumour–host relationship* was also estimated in terms of a 1–4 point scale by the mode and stage of invasion and the degree of lymphoplasmocytic infiltration, considered as a possible sign of an inflammatory reaction or a local immunological reaction (Table II)

The six morphological parameters thus permit a grading with a total points ranging from 6–24

The application of the grading system to different morphological parameters is illustrated for the tumour cell population in Figs 1 and 2 and for the tumour–host relationship in Figs 3 and 4 Fig 1 shows a highly differentiated tumour exhibiting fairly large cells with rounded, indolent nuclei in a monomorphic pattern and with almost no mitosis (point scale 1) Fig 2 gives an example of a cancer, point scale 4, illustrating the cellular structure of an invasive, low differentiated carcinoma with a sarcomatous pattern, large hyperchromatic cells and numerous mitoses In Fig 3, well defined cords of a cancer are superficially penetrating into the stroma, surrounded by heavy lymphoplasmocytic infiltration, point scale 1 Fig 4 illustrates a tumour with a sarcoma like pattern, heavily infiltrating the stroma and with scanty lymphoplasmocytic infiltration, point scale 4 The examined cases were found to fall within the range of 8–24 points

Table II *Histological grading of malignancy*

	II Tumour–host relationship			
	1	2	3	4
Mode of invasion	Well defined borderline	Cords less marked border line	Groups of cells, no distinct borderline	Diffuse invasion
Stage of invasion	Susp	Micro carcinoma few cords	Nodular invasion connect tissue	Massive invasion
Cellular response	Marked	Moderate	Slight	None

Fig. 1. Tumour cell population, point scale 1. A highly keratinised area (*upper left*) a few enlarged nuclei and single mitoses. Photomicrograph, HE, × 400

Fig. 2. Tumour cell population, point scale 4. Poorly [illegible] anaplastic differentiation with an anaplastic, immature enlarge nucleus and numerous mitoses. HE, × 400

Fg 3 Tumour host ationsh p po nt scale I Sol d cords with well def ned border l nes and marked cellular response Photom crog aph HE, 150

Fig 4 Tumour host relat onsh p po nt scale 4 Cancer with d ffuse inf ltrat on mass vely invad ng the under ly ng stroma with hardly any cell lar response I hotomicrograph HE, 150

Table III *Clinical stage and histological grade of malignancy in 124 cases of carcinoma of the gingiva (total number of patients diagnosed at the ENT department, Karolinska sjukhuset, during 1958–68, 12 years)*

Histol grade of malignancy		Clinical stage			
Group	Points	T1	T2	T3	T4
A	<14	8	10	16	10
B	14–16	4	0	20	21
C	>16	5	2	13	15

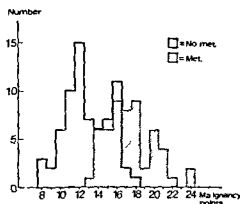


Fig 5 Frequency of individual cases with and witho metastases expressed in total points value

RESULTS

The clinical stage (T1–T4) and histological grade of malignancy according to the point system are demonstrated in Table III The material was divided into three groups Group A with no metastases except for one patient, group B with or without metastasizing tumours (overlapping group) and group C which had metastasizing tumours except for 2 heavily treated patients From the Table III it is evident that no definitive correlation exists between the clinical stage and the histological grade of malignancy A dency to a relatively greater number of cases clinical stage T3 and T4 was found among umours with a high grade of malignancy (28 out of 35 in group C compared with 26 out of 44 in the group A)

The distribution of the number of patients with or without metastases expressed in total point value is demonstrated in Fig 5 The distribution of the frequency of individual cases with metastases, expressed in terms of total point values, showed a shift towards higher poi values Within the total material 54 patier evidenced metastases and 70 did not Of patients with metastases, 84% had their met stases already on admission to hospital and 16 had developed metastases later in the course the disease Two patients with high malignan points and no metastases were found in tl material, the clinical course as well as the trea ment are tabulated in Table IV

Patients undergoing surgery alone are tabula ted in Table V In group I, small cancers usuall scored low malignancy points and subsequentl had a good outcome Patients with recurrence within group 1 had point scale 3 or 4 in th parameter "mode" or "stage of invasion Group 2 showed 3 low point tumours two o them with recurrences The latter two were al point scale 3 or 4 in the parameter state invasion Group 3 showed 3 high point tumour and these as well as other high point tumou within group 1 or 2 has a poor outcome Th

Table IV *Clinical course of patients with "high point"-tumours without metastasis*

Patient no	Malignancy points	Stage	Course	Treatment
30	17	T3	1 c 7 years no cancer	6500 R
48	17	T4	1 c 8 years reticulum cell sarcoma no cancer	6000 R + submand exc + local exc

Table V. *Patients with gingival carcinoma, primarily treated with surgery*

Clinical stage	Patient no	Surgery performed	Total malignancy points	5 years alive No recurrence
I (T1 + T2)	116	(1) Local excision (2) Local excision + rad neck diss	8	Local recurrence after 1 year Symptom free 5 years after secondary surgery
	24	Local excision	10	Yes
	85	Local excision	10	Several local recurrences Symptom-free 5 years after secondary surgery
	75	Local excision	11	Yes
	96	Local excision	11	Local recurrence after 9 years
	83	Local excision	12	Yes
	88	Local excision	12	Yes
	97	Local excision	12	Local recurrence after 1 5 years Dead of suicide "Mode" and "stage of invasion" point scale 3
	84	Local excision	15	*Local recurrence after 7 years* Symptom free and alive 5 years after secondary surgery
II (T3 + T4)	41	Hemimand -ectomy + local excision	12	Local recurrence after 2 years, still alive after 5 years "Stage of invasion" point scale 3
	111	(1) Gum commando (2) 6 500 R	12	*Local recurrence after 3 years* Symptom free 3 years after irradiation
	68	(1) Local excision + Lig A car ext (2) Local excision	13	Local recurrence after 2 years but symptom free 9 years after secondary surgery
	53	(1) Local excision (2) 6 000 R	14	Local recurrence Dead in cancer after 2½ years
	108	Gum commando	14	Dead in cancer after 1 year with pulm met
	113	(1) Gum commando (2) 6 200 R	16	Local recurrence after 1 year Dead in cancer 3 years after secondary radiation
III (N+)	91	Gum commando	13	Symptom free after 9 years
	39	(1) Local excision (2) Local excision + hemi mand -ectomy + ext submand	14	Local recurrence after 2 years Dead in cancer after 3½ years
	67	Gum commando	16	Dead in hypofarynxcarcinoma 7 5 years later
	90	(1) Gum commando (2) 4 000 R + local excision + partial neck diss contralat side	17	Local recurrence + sec met after 1 year Dead in cancer 1½ years later

only low-point tumour within group 3 had a good outcome

Analysis of the distribution of patients according to points within different parameters is set out in Table VI In the group with no metastases, the tumours were more highly differentiated, the mitosis rate was as expected low, but nuclear polymorphism was sometimes quite advanced The tumours sometimes had a diffuse growth pattern but mostly grew in solid cords with sharp boundaries However, 4 patients received point scale 4 in this parameter Many patients had an advanced state of penetration but nevertheless no metastases took place The

Table VI *Analysis of frequency of patients within the different point scale groups*
Material divided into metastasizing group (54 pats) and non met group (70 pats)

Parameter	Non metastasizing group (70 pats) 1	2	3	4	Metastasizing group (54 past) 1	2	3	4
Tumour cell population								
Differentiation	29 (41 %)	34 (49 %)	7 (10 %)	– (0 %)	4 (7 %)	23 (43 %)	19 (35 %)	8 (15
Nuclear polymorphism	19 (27 %)	28 (40 %)	23 (33 %)	– (0 %)	1 (2 %)	18 (33 %)	26 (48 %)	9 (17
Mitoses	36 (51 %)	23 (33 %)	11 (16 %)	– (0 %)	8 (15 %)	25 (46 %)	17 (31 %)	4 (7
Tumour–Host relationship								
Mode of invasion	2 (3 %)	33 (46 %)	31 (44 %)	4 (7 %)	– (0 %)	2 (4 %)	16 (30 %)	36 (67
Stage of invasion	– (0 %)	10 (14 %)	44 (63 %)	16 (23 %)	– (0 %)	– (0 %)	12 (22 %)	42 (78
Cellular response	27 (39 %)	31 (44 %)	12 (17 %)	– (0 %)	10 (19 %)	20 (37 %)	19 (35 %)	5 (9

cellular response was as a rule intense in this group

In the metastases group, the tumours were much more low differentiated, and showed advanced nuclear polymorphism with an enhanced mitosis rate The tumours as a rule had a diffuse growth pattern and showed an advanced state of invasion but the cellular response seemed to be more even distributed over the point scale groups

Dedifferentiation of the tumour during the urse of the disease was rarely found in the terial Thus the histological appearance was nerally unchanged in recurrences, as compared with the tumour before treatment

Patients with multiple carcinomas of the oral cavity generally exhibited similar structur which may reflect an identical "fingerprin response to the same possible carcinogenic influence

SURVIVAL DATA

The survival rates following 3–15 years' follow up in terms of patients dead of cancer, dead of intercurrent disease within or after 5 years, respectively, as well as those alive, are illustrated in Fig 6 Of the 124 patients, 89 were dead, 73 from cancer and 16 from intercurrent disease 8 within 5 years and 8 after 5 years of disease Thirty five patients were alive following 3–15 years' follow-up A total of 110 patients were followed for more than 5 years The relation between degree of histological malignancy ex pressed in total malignancy points and number

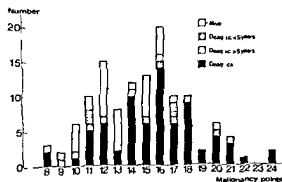


Fig 6 Frequency of individual cases alive dead inter

values

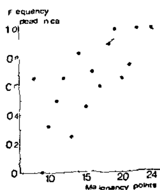


Fig 7 Frequency of patients dead of cancer A close correlation exists between death from cancer and higher point scale tumours

of patients dead of cancer is illustrated in Fig 3 From the figure it is obvious that a close correlation exists between the degree of histological malignancy and a fatal outcome of the disease A tumour with a point value of 16 will almost always lead to the death of the patient

DISCUSSION

Cancer of the gingiva is a fairly rare disease In south India, however, it constitutes roughly 9% of all cancer cases seen (Krisnamurthi & Shanta, 1963), in the United States roughly 1% (Martin, 1941, Cady & Catlin, 1969) and in Sweden 0 5% (*Cancer Incidence in Sweden 1958–1969*) Twenty-five percent of all oral cancers in south India, (Krisnamurthi & Shanta, 1963), 12–16% in the United States (Mattick & Meehan, 1951, Wilkins & Vogler, 1957) and 7% in Sweden (*Cancer Incidence in Sweden 1958–1969*) are squamous cell cancers of the gum During the years 1958–69 358 new cases were registered in Sweden (*Cancer Incidence in Sweden 1958–1969*) The disease affects mostly older persons, and offers many therapeutical problems Opinions vary widely regarding treatment It is therefore of the utmost interest to reach an optimal classification of the squamous cell carcinoma, so that adequate therapy can be instituted at an early stage of the disease

Prognoses and given treatment in this material have been analysed and published elsewhere (Nathanson et al, 1973) Surgery alone and combined treatment have both been applied Moreover, many different forms of radiotherapy have been applied, which breaks down the radiotherapy groups to such an extent that no correlation according to malignancy has been carried out, except for two cases These patients received a heavy radiation therapy and one radical surgery as well which may explain the cure of these patients (Table IV)

The group undergoing surgery alone is analysed in Table V The findings in the present material show that the best results from treatment of gingival carcinoma stage I (T1+T2) are achieved with surgery alone (in agreement with earlier findings, Martin, 1941) Moreover, the special verrucose form in locally aggressive non-metastasizing epidermoid carcinoma, in this type of classification receiving very low total malignancy points, has been reported to undergo anaplastic transformation following radiation therapy (Demian et al, 1973) Small cancers (T1+T2) with a low total malignancy point should therefore be treated with surgery only

As seen from the results in this study, the tumours are more highly differentiated at the surface and less differentiated in the invasive, deeper portions This means that an adequate estimation of the biological activity of the squamous cell carcinomas will fail, if the tumour is merely classified as highly, moderately, or poorly differentiated and if the biopsy is taken more superficially It is therefore essential to classify the tumour cell population separately from the growth characteristics, revealed by the relation of tumour to the adjacent tissue component

The histological classification system used in this study gives a grading from 1–4 of a total of six morphological criteria Each of these parameters is an indicator of the degree of malignancy Although some of the parameters paralleled each other to some extent, e g mode of invasion and nuclear polymorphism, this was not always the case Several papillomatous, keratinizing tumours with invasive growth in the form of minute cords could also show considerable nuclear polymorphism, which rendered a higher point value than would be the result of an estimation of the degree of differentiation alone It is thus of great value to evaluate as many relevant morphological criteria as possible A point scale estimated in such a way seems to serve as a better measurement of the biological activity, than would be the case for a single criterion

The analyses of the different parameters and their significance for metastases as well as for survival gave some remarkable findings As a rule, nuclear polymorphism, mode of invasion, and total malignancy points were the best parameters to forecast the clinical outcome of the

disease This is in agreement with earlier findings (Jakobsson, 1973)

However, in the group without metastases the nuclear polymorphism was quite advanced in 4 patients, having point scale 4 Analyses of those 4 patients showed that 2 of them belonged to the high treatment group shown in Table IV The other two had very low sums of points for the tumour cell population The cellular response was very intense in the group without metastases and thus seems to be a sign of benignancy, though the exact mechanism involved is not known There are several reasons for assuming that the total point values in the individual cases, of dead in cancer, showed an obvious correlation with high malignancy points

It was found in this study that even indolent cancers can sometimes kill Many of the patients are at an advanced age and others come to the clinic with a cancer in an already advanced state

Almost all patients with metastasizing tumours have their metastases already on admission to hospital Health control, careful examination of the oral mucous membrane at routine dental examination, and strict "carcinoma in situ" ...sties may perhaps reduce the figure Be-...se of the dynamic in this high malignancy cell ...lation, a more cautious attitude is indicated, ...nalogous to what has been reported for some types of cancer in cervix uteri, exploding into invasive cancer within a few months (Ashley, 1966)

The histological grading of malignancy presented here implies a good correlation to the survival rate Thus it would seem possible to diagnose squamous cell carcinomas of the gingiva in biopsies from the primal tumours and to determine an adequate therapy at an early stage of the disease Moreover, many new approaches for further research work on gingival cancer have been opened up

ZUSAMMENFASSUNG

Eine histologische Klassifikation und Graduierung der Malignität der primären Biopsien von 124 Patienten mit Plattenepithel Karzinom der Gingiva, behandelt während der Zeit von 1958–1969 in Karolinska Sjukhuset, ist durchgeführt worden Sechs unterschiedliche morphologische Charakteristika wurden bewertet ausgehend vom Aussehen der Zellen und Tumor Wirt Verhältnis, und einer Skala von 1–4 zugeordnet Dies erlaubte eine Graduierung von total 6–24 Man fand eine starke Beziehung zwischen dem Grad der histologischen Malignität und dem tödlichen Ausgang der Fälle So führte ein Tumor mit einem Wert von 16 Punkten fast immer zum Tod des Patienten während die besten Resultate rein chirurgisch in der Gruppe kleine Karzinome (T1 + T2), mit niedrigen Malignitätspunkten erreicht wurden

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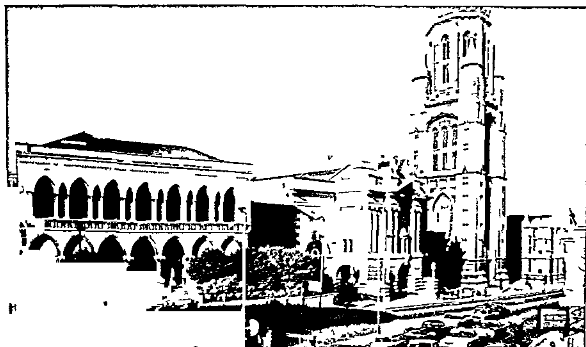
COMPTE RENDU
DE LA RÉUNION SCIENTIFIQUE DU

COLLEGIUM OTO-RHINO-LARYNGOLOGICUM

AMICITIAE SACRUM

BRISTOL, LE 8–11 SEPTEMBRE, 1974

Almqvist & Wiksell, Uppsala 1975



PRESIDENT'S ADDRESS

Mesdames et Messieurs,
nous sommes tres heureux de vous accueillir dans cette ancienne cite de Bristol pour le congres du Collegium Nous esperons que votre visite sera aussi agréable que profitable

Meine Damen und Herren,
ich heisse Sie herzlich willkommen in Bristol Wir sind hoch erfreut, Sie bei uns zu sehen Wir hoffen, dass Sie Ihren Aufenthalt hier voll geniessen und eine angenehme Erinnerung mit nach Hause nehmen werden

I understand that for many of you this is your first visit to this historic and beautiful city which has played an important part in the history of England, and indeed in the development of our modern civilisation This has been a city of pioneers in exploration, in business, in science and in medicine Three are illustrated on your programme First the Cabot Tower commemorating John and Sebastian Cabot s discovery of the mainland of North America in 1497 Second Brunel s Suspension Bridge, erected over one hundred years ago He also built the railway from London to Bristol and the Steamship Great Britain", the first propeller driven ship to cross the Atlantic Finally, the Concorde, the engines of which are built in Bristol

Our teaching hospital founded on the Bristol Royal Infirmary, was first built in 1734, and is the oldest provincial hospital in England Earlier still, in 1395, the Barber Surgeons were established in Bristol, and took part in civic processions and trained apprentices

St Peter s Hospital, the first Poor Law hospital, was established in the Aldworth Mansion in 1698 Dr Dover was its first physician, famous for his introduction of Dover s Powder (Pulv Ipecac Co) and also for his discovery and rescue of Alexander Selkirk from the Island of Juan Fernandez He had sailed with Woode Rogers in two vessels, the *Duke* and *Duchess*, on a privateering voyage around the world He

MIDDLE EAR SPECIFIC PROTEINS IN GLUE EARS

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Abstract Two rabbits were immunized with a pool of mucoid secretion from glue ears The ear fluids formed up to three specific protein precipitation lines with the absorbed immune serum One of these proteins was identified as containing acid and another as containing neutral glycoproteins, the third component remained unidentified The data bring out new evidence of the active secretory capacity of the middle ear mucosa in secretory otitis media

In earlier reports we (Palva et al, 1974*a*, *b*) have shown that, in mucous ear fluid, the activity of acid phosphatase, lactate (LD) and malate dehydrogenases (MD) and of aminotransferases was significantly greater than in serum This [illegible] was so large that active secretion [illegible] be assumed, since simple filtration from capillaries could not possibly increase enzyme activity in secretions up to 30 times above the serum concentration The LD isoenzyme pattern in glue ear fluids was also identical with that demonstrated in homogenized middle ear mucosa (Palva et al, 1970) As for MD, two isoenzymes were found in ear fluids whereas sera from the patients revealed only one Nonspecific esterases, which had lower activity in ear fluids than in serum, showed one isoenzyme in the ear fluid, a tissue esterase, which was absent from serum (Palva et al, 1974*a*)

Increased activity of acid phosphatase, LD and MD was also reported by Juhn et al (1971) in serous ear fluids although the difference as compared to serum was not nearly so marked as in our glue ear series Later Juhn et al (1973) found that the LD isoenzyme pattern in serous fluids was similar to the one observed by us in the mucosa and the mucoid secretion

Specific proteins possibly secreted by middle ear mucosa were sought for by Tonder & Gundersen (1971) using rabbit middle ear fluid antisera However, they found no evidence of specific proteins and concluded that the ear fluid mainly was of serum origin

Recently Bernstein et al (1972) demonstrated a precipitation line with colostrum and middle ear fluid when an IgA secretory component specific antiserum was placed adjacent to these secretions, but no line was demonstrable with the patients' serum A similar result was obtained with an antilactoferrin serum They also quoted Tomasi as reporting two new antigens from the amniotic fluid, the antisera being specific only for amniotic an middle ear fluid but not for plasma

MATERIAL AND METHODS

The middle ear fluid specimens were obtained i connection with aspiration and insertion of ventilating tube All represented the typica thick secretion of the glue ear

Immunization of two rabbits was carried ou with a pool of fluid specimens from glue ear using a dilution containing 10 mg of protein/ml One ml of this dilution was emulgated with 1 m of Freund's adjuvant complete and thus, in each injection, both rabbits received 5 mg of protein Immunization was performed intracutaneously 4 times at 4-week intervals Five days after the fourth injection a blood sample was drawn for preliminary immunoelectrophoretic analyses As these were not yet con

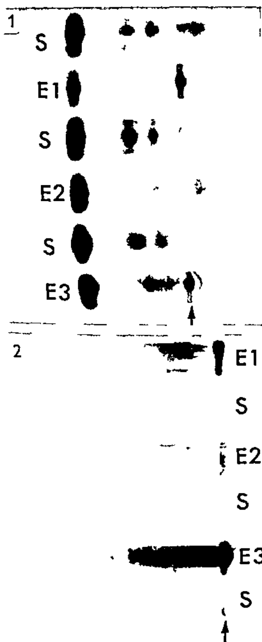


Fig 1 Electrophoretic protein analysis of three sera and ear fluids on cellulose acetate Serum (*S*) is undiluted ear fluids (*E*) diluted 1 in 10 The same fractions of varying strength appear in both fluids The application point is marked by an arrow

Fig 2 Electrophoretic analysis of acid glycoproteins in the sera and ear fluids stained by Alcian blue Serum (*S*) shows no staining the ear fluid (*E*) contains one part of the positive material at the application point and another migrating towards the anode in diffuse bands of varying length

sidered good enough, a booster was given Six days later the rabbits were bled by the earlobe and 30 ml of blood was drawn for the final immunological studies

The antibodies against human serum proteins were absorbed from rabbit anti middle ear fluid serum with freeze-dried human serum proteins The completeness of absorption was tested by immunodiffusion and immunoelectrophoretic techniques

Electrophoretic protein analyses were made using films of cellulose-acetate and Beekman's Michozone apparatus For protein staining Ponceau S stain was used, acid glycoproteins demonstrated by Alcian blue (AB) and neutral by periodic acid Schiff (PAS) stains

Immunoelectrophoretic and immunodiffusion analyses were performed using celluloseacetate and agarose gels A microdiffusion method (Wadsworth, 1957) modified by Krause & Raunio (1967) was employed The activity of acid and alkaline phosphatases, of LD and MD, and of nonspecific esterases were determined as reported earlier (Palva et al , 1974*a*)

Acid glycoproteins were precipitated from 1 to 10 dilution of glue ear fluids with 5% aqueous cetylpyridinum chloride The precipitate was dissolved in a small volume of 0 9% NaCl Electrophoresis runs and immunodiffusion tests were carried out also from this solution

RESULTS

Protein analysis

Fig 1 shows typical examples of serum and ear fluid analyses on cellulose acetate on three patients The albumin fraction was strong in all cases, while in all other areas there were bands that varied in strength Neither with cellulose acetate nor with agarose could any bands be demonstrated which were specific for either fluid

Alcian blue staining

An entirely different picture was obtai
AB-staining (Fig 2) The serum e'

were devoid of AB positive material while the ear fluid runs showed much AB positive material to be present A good amount of these acid mucopolysaccharides remained at the application point while a tail like continuous glycoproteins band moved anodically and extended to the region of α globulins This was the standard finding in all cellulose acetate electrophoretic analyses No test could be made on agarose which despite purification contains acid groups

PAS staining

A somewhat similar result for ear fluid was seen with PAS staining as some of the applied fluid stained in and did not move from the application point (Fig 3) A weaker PAS-positive tail than seen for AB moved anodically In the sera, PAS positive material was demonstrated, one band in the region of α_1-globulins and another at the application point

Immunodiffusion tests

In all macroimmunodiffusion tests, the ear fluids constantly showed one precipitation line against rabbit middle ear fluid antiserum In ~ e middle ear fluids, two precipitation arcs 1 (Fig 4) Several tests with human saliva consistently negative (Fig 5) In the microimmunodiffusion tests, two precipitation lines were seen in two fluids and three in one ear fluid (Fig 6) All these precipitation lines stained positive with Amidoblack, and one with PAS stain

One AB positive line could be demonstrated in immunodiffusion on cellulose-acetate very close to the well containing the ear fluid This line was weak and did not appear in all ear fluid specimens (Fig 7)

Immunodiffusion tests with alkaline and acid phosphatases, LD MD and nonspecific esterases were all negative

Immunoelectrophoresis

One distinct precipitation line always formed between the well containing the ear fluid and the longitudinal trough filled with anti middle ear fluid serum As in the immunodiffusion tests,

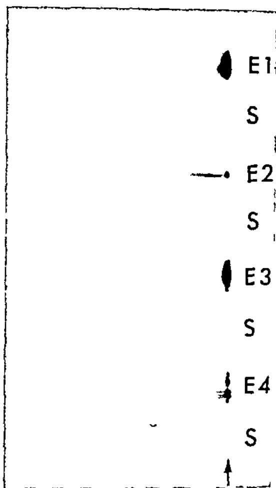


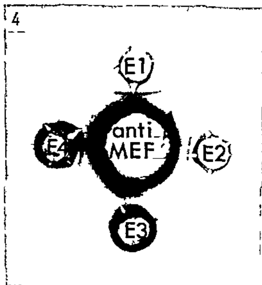
Fig 3 PAS staining of neutral glycoproteins in fou sera (*S*) and ear fluids (*E*) The serum shows PAS positive material in the region of α_1 globul ns and at th application point In the ear fluid most of the pos tiv material remains at the application point a smaller portion migrates anodically in diffuse fashion

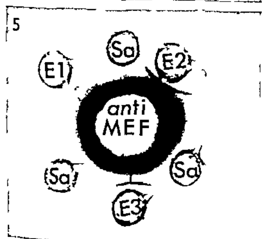
the ear fluids in some specimens showed another, weaker line (Fig 8) Tests with saliva were always negative *PAS staining in immunoelectrophoresis* showed that the regularly appearing stronger precipitation line stained bright red with PAS (Fig 9)

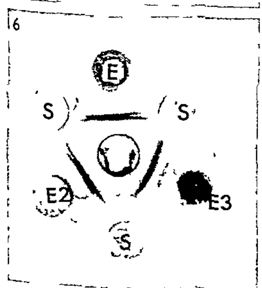
Treatment of the ear fluids with cetylpyridinum chloride did not result in any new observations

DISCUSSION

The present results bring out two important points First, a large amount of acid glyco-







proteins appear in ear fluid but are absent from serum Secondly, up to three specific protein precipitation lines may appear with middle ear fluid, one of them consists of a protein bound to neutral glycoproteins and stains with Amidoblack and PAS One protein line remains unidentified for the time being A third middle ear specific protein contains acid glycoproteins and stains with AB This last protein precipitate scarcely moves from the application point, whereas the other two show a better mobility

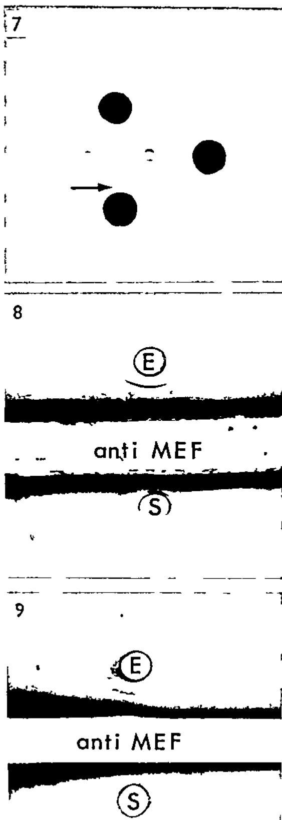
The negative results with the five enzymes tested were to be expected since the antigenic properties of these enzymes cannot be different in ear fluids and in sera

The present data show convincing evidence that specific substances are secreted into the middle ear fluid by the mucosa itself With the supporting evidence of Bernstein et al (1972) and of Mogi et al (1973), as to the presence of the secretory piece IgA in middle ear fluids there is no longer any doubt about the active part played by the middle ear mucosa The precipitation lines formed by our antisera do not perhaps represent secretory piece protein since no precipitation lines were formed with saliva Furthermore the location of secretory piece precipitate in immunoelectrophoresis (Brandtzaeg et al , 1970) is different from those proteins demonstrated in the present study

Fig 4 Immunodiffusion test on agarose gel The central well contains rabbit anti middle ear fluid serum (anti MEF) and the surrounding wells four different middle ear fluids (*E*) The fluid in the lowest well forms two precipitation lines with the antiserum, the other three fluids show only one line

Fig 5 Immunodiffusion test with saliva (*Sa*) in every second well No precipitation lines forms with saliva the ear fluids show one line each

Fig 6 Immunodiffusion test using the micromethod on agarose gel Serum (*S*) in every second well produces no precipitation lines while at least two of the ear fluids (*E*) form three lines with antiserum and the third ear fluid on right forms two, perhaps three, lines Amidoblack staining



ZUSAMMENFASSUNG

Zwei Kaninchen wurden mit mukosem Mittelohrsekret, das von Fällen mit Otitis media sekretoria stammte immunisiert Drei spezifische Präzipitatlinien wurden anhand des absorbierten Immunserums aufgezeigt Eines dieser Proteine enthielt saure und ein anderes neutrale Glykoproteine, während man das dritte nicht identifizieren konnte Die Befunde zeigen dass die Mittelohrschleimhaut eine aktive Rolle bei der Bildung des mukosen Mittelohrsekrets spielt

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Fig 7 Immunodiffusion test with three ear fluids on cellulose acetate stained by Alcian Blue One fluid forms a distinct precipitation line very close to the well (*arrow*)

Fig 8 Immunoelectrophoresis on agarose gel The central trough contains antiserum (anti MEF) The top well is filled with ear fluid (*E*) and the bottom well with serum (*S*) Two distinct precipitation lines form with the ear fluid

Fig 9 Immunoelectrophoresis PAS staining The stronger precipitate shown in Fig 8 stains positively and therefore contains neutral glycoproteins

Wadsworth, C. 1957 A slide microtechnique for the analysis of immune precipitates in gel *Int Arch Allergy Appl Immunol 10*, 350

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DISCUSSION

H H Naumann. The mucosa of the middle ear seems to be similar to that of the nose In the nasal mucous membrane we know some details about the IgA IgE and IgM and also about some protective enzymes, like α_1 antitrypsin and other inhibitors. My question is You spoke about three special proteins you could detect. Is there any evidence that your special proteins have something to do with any kind of immunoglobulins and or an imbalance of inhibitors or other protective enzymes?

I Friedmann. Congratulations on his Jennerian stubbornness" in elucidating some add tional biochemical details of secretory otitis Question do these findings confirm the secretory character of the mucosa in such patients?

J Sade We know that there are three sorts of proteins present in the glue if a glue ear secreted in situ

1 Glycoproteins demonstrated histochemically or being secreted by globlet cells, which are the mechanical back bone of mucus.

2. Immunoglobulins (shown by Nagi and by Bernstein) playing an immunological role.

3 The various enzymes you so nicely showed—do you know what role they play? Could they be a result of leucocyte breakdown—leucocytes being always present in the middle ear effusion?

T Palva (Reply) to Mr *Naumann.* It is obvious that tympanic mucosa secretes substances into the tympanic cavity basically in the same way as the maxillary mucosa does. We have not yet studied quantitatively the immunoglobulins but have recently been able to show that the IgA secretory piece is present in middle ear fluid and is the fourth of the specific mucosally secreted proteins found by us.

To Mr *Friedmann.* The answer is yes and you have yourself demonstrated beautifully the development of secretory otitis in guinea pigs.

To Mr *Sade* Your questions are difficult to answer At the present stage my feeling is that the high activity of enzymes in the middle ear mucosa represents the active metabolism of the secretory system, possibly also in anaerob c conditions as suggested by the LD isoenzyme pattern

Acta Otolaryngol 79 166–171, 1975

MORPHOLOGISCHE BEFUNDE ZUR IMMUNBIOLOGIE DES KEHLKOPFKREBSES

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Abstrakt An Hand einer Klassifizierung der Stroma reaktion des Kehlkopfkrebses und der Bilder aus den regionaren Lymphknoten wird Leistung und Ablauf der korpereigenen Abwehr in immunbiologischer Sicht dargestellt Wir unterscheiden Reaktionstypen wie chronisch-entzundlich, epitheloidzellig tuberculoid und aggressiv am Stroma des Primartumors und ordnen Sinusreaktionen an den Lymphknoten zu Letzteres scheint der Gipfel korpereigener Leistung zu sein, da bei fortgeschrittenem Krebsleiden kaum mehr beobachtbar Die morphologischen Befunde lassen prognostische Schlusse zu da Patienten mit aggressiver Stromareaktion und ausgepragter Sinusreaktion funf Jahre und langer den operativen Eingriff überleben

Reaktive Lymphknotenveränderungen bei Fruhfällen von Carcinomen nach Black & Speer (1960), die Klassifizierung der Stromareaktion [illegible] Krebs nach Ratzenhofer (1970), die [illegible]ndzellige Reaktion nach Wuketich (1959) [illegible] die aggressive Stromareaktion nch Ratzenhofer (1970) lassen die Annahme einer immunbiologischen Beziehung zwischen Geschwulstträger und Tumor aus morphologischer Sicht zu In Fortfuhrung eigener Untersuchungen die uber Anregung H Mosers schon auf die Jahre 1962/63 zuruckgehen, ermutigt durch das Verhandlungsthema Fortschritte der experimentellen und klinischen Tumorforschung, deutscher *HNO Ärzte 1973 haben wir versucht, morphologische* Daten fur ein immunbiologisches Geschehen beim Kehlkopfkrebs aus operativ gewonnenem Material zu finden

Stromareaktion und das Bild regionärer Lymphknoten aus dem Abflußgebiet des Primärtumors haben wir im Laufe von zehn Jahren an mehr als hundert Operationspraparaten untersucht Wir sichteten ähnlich wie Paavolainen (1970) die Stromareaktion nach den Ratzenhofer'schen Kriterien Charakter der *zelligen Infiltration, Strukturen und Verhalten* des proliferierenden Krebsparenchyms Darüber hinaus verglichen wir diese Befunde mit der Morphologie der Lymphknotenreaktion, wie von uns bereits fruher mitgeteilt

Die *chronisch entzündliche Reaktion*, welche als Reizantwort auf Irritation jeder Art, aus lymphoplasmo leukozellulären Infiltraten, Vermehrung ortsständiger Bindegewebszellen sowie entzündlicher Exsudation, aus verstärkter Durchblutung besteht, ist auch bei praecancerösen Veränd*erungen an der Schleimhaut zu sehen Immer* aber ist sie Vorläufer oder Begleiter der beginnenden Invasion Beim manifesten Krebs ist sie wechselnd stark ausgebildet vom Krebsparenchym mehr minder deutlich abgesetzt, welches sich deshalb auch als kaum alteriert erweist Im regionären Lymphknoten fanden wir den praemetastatischen Sinuskatarrh und die Sinushistiozytose, von uns Sinusreaktion I bezeichnet Das Zellbild in den machtig erweiterten Sinus ist geprägt von der Proliferation der Sinusendothelien, der Mastozytose, der Plasmazelldichte im angrenzenden Mark, den Veränderungen am Fasernetz wie Aufbruch *und Verklumpung, sowie* den vasculitisähnlichen Gefäßveränderungen Diese Veränderungen sind an Rand und Intermediarsinus zu *sehen als Zeichen eines im Übermaß angebotenen* Abbaumaterials, welches sicherlich teilweise auch am Blutweg angeboten wird

Die *epitheloidzellige tuberkuloide Reaktion* gekennzeichnet durch Phagozyten, Epitheloid zellen und Riesenzellen in Granulomform angeordnet, sahen wir mit und ohne Krebszellresten



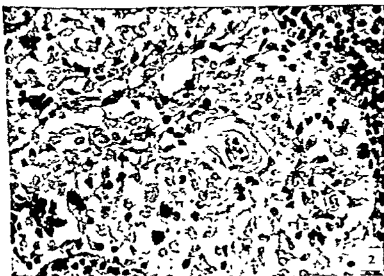




Abb 1 Sinusreaktion mit weiten zellreichen Sinus, HE gefärbt, Übersicht.
Abb 2 Sinusreaktion mit Mastzellen, Histiocytose und vasculitisähnlicher Gefäßreaktion Giemsa gefärbt
Abb 3 Sinusreaktion mit Faseraufbruch u Verklumpung. Versilberung.

Wie Ratzenhofer im Stroma und Wuketich im regionären Lymphknoten eines Karzinoms, fanden wir sie, an Fremdkörpergranulome erinnernd, einzeln stehend, aber auch dichtstehend wie bei Sarcoidose. Molekuläre Zerfallsprodukte scheinen für ihre Entstehung verantwortlich, insbesondere fetthaltige Zerfallsprodukte der Krebszellen, sogenannte Malignolipide. Wir sahen sie im Lymphknoten immer dann, wenn durch Nekrobiose im Primärtumor devitalisiertes Material im regionären Lymphknoten angeboten wird. Kosaki u. Mitarbeiter (1958) *sprechen von einer Antigen-Antikörperreaktion* gegen Phospholipide. Nadel & Ackermann (1955), sowie Neumann & Hommer (1950) deuten diese Reaktion sogar als Zeichen einer Abwehr. Wir meinen wie Ratzenhofer daß jedoch die Resorption im Vordergrund steht und möchten zur Stützung dieser Auffassung eine weitere Beobachtung anführen. Wir sahen in Lymphknoten auch an Cholesteringranulom erinnernde Herde, die ein sicherer morphologischer Beleg für Verarbeitung stark lipoidhaltiger Stoffe sind. Sie lagen den Marksinus entsprechend und in ihrer Umgebung fanden ch einzelne Riesenzellen im Parenchym des hknotenmarks.

aggressive Reaktionstyp ist geprägt durch granulationsgewebsartigen zellreichen Stromagürtel nach Ratzenhofer um Krebsverbände, welche in ihren virulenten Randzonen nekrotisch *und anschließend abgebaut werden.* Neben überwiegend Lymphozyten und Plasmazellen beherrschen große mononucleäre Histiozyten das Zellbild. Gerade diese Zelltype scheint *Träger der zellständigen Abwehrleistung im* Stroma des Primärtumors und im regionären Lymphknoten zu sein. Als Sinusreaktion II haben wir diese aktive Proliferation ehemals bezeichnet. Diese Aggression vitaler Krebsnester im Stroma und Lymphknoten gipfeln in bindegewebigen auch hyalinisierten Narben als Ergebnis umschriebener Heilung. Trotz Untersuchung ganzer Halsbindegewebeblöcke und der in Stufenserie geschnittenen Primärtumoren, waren solche Befunde selten. Sie waren stets lokal begrenzt auf einzelne Krebszellnester, nie an allen Krebsmetastasen gleichzeitig beobachtbar. Oettgen (1973) hat auf Grund vorwiegend serologischer Untersuchungen von der Immunbiologie des Krebses gesprochen. Mosers Untersuchungen über Intracutanreaktionen beim Karzinomkranken sind ein stets reproduzierbarer Beleg dafür. Immunreaktionen können vor Krebs schützen, sie können den Ablauf verzögern und bei Insufficienz beschleunigen. Ratzenhofer nannte seine Befunde Ansätze einer Krebsheilung. Jede histomorphologische Untersuchung liefert nur eine Momentaufnahme *eines immunbiologischen Geschehens, in dessen* Zentrum die Krebsgeschwulst steht und deren Träger die körpereigene Abwehr ist. Aus unserem Material konnten wir einen Ablauf und typische Verhaltensweisen der Erkrankung herauslesen, besonders deutlich am Lymphknoten. Die Sinusreaktion I ist der Beginn der Auseinandersetzung, induziert durch die chronisch entzündliche Stromareaktion bzw. als Antwort auf eine Irritation oder Noxe aus dem Einflußgebiet. Höchstleistung der Resorption spiegelt sich in der epitheloidzelligen Reaktion und Bildern ähnlich dem Cholesteringranulom. Die aggressive Stromareaktion und die Sinusreaktion II sind eine Folge und der Gipfel der körpereigenen Abwehrleistung. Eine regelmäßige Beobachtung, daß sich beim Angehen der ersten Krebsmetastasen das Lymphknotenbild ändert, ist ein weiterer Beleg. Die weiten zellreichen Sinus *schwinden. Die Krebszellnester liegen in Lymph*knoten mit mäßiger follikularer oder lymphatischer Hyperplasie und scheinen völlig unberührt. Solche Befunde bedeuten an unserem *Material schlechte Prognose.* Die Patienten verstarben innerhalb eines Jahres post operationem, nicht selten an Fernmetastasen. Hingegen steigen die Überlebenschancen mit dem Grade und der Ausbreitung der Sinusreaktion. Patienten mit tuberkuloider oder aggressiver *Stromareaktion und Sinusreaktion I und II* überleben 5 Jahre und länger.

Wenn also der Lymphknoten einerseits ähnlich wie die Stromareaktion eine Barriere gegen den Krebs darstellt und einer der Orte des immunbiologischen Geschehens ist, wie sind





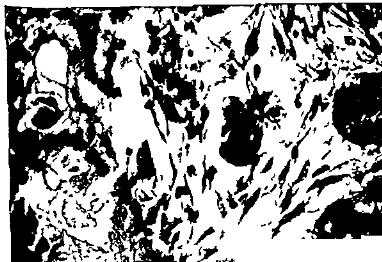


Abb 4 Epitheloidzellig tuberkuloide Reaktion im Lymphknoten im Zentrum untergehende Krebszellreste HE gefärbt
Abb 5 Cholesteringranulom im Lymphknotensinus HE gefärbt.
i Aggressive Stromareaktion
icht HE gefärbt

dann die Halslymphknoten in unsere therapeutischen Bemühungen beim Kehlkopfkrebs einzubeziehen? Gusič hat diese Gedanken immer wieder bei der prophylaktischen Hals räumung ausgesprochen Auch wissen wir aus dem eigenen Material, daß nach Neckdissection die Fernmetastasenrate steigt Unsere Lymphknotenuntersuchungen zeigen aber Mikrometastasen in Knoten, die dem Regionaritätsprinzip widersprechen, möglicherweise hämatogen angegangen sind Der Kehlkopfkrebs hat weiters seine Häufigkeitsspitze jenseits der 5 Lebensdekade nach Schmid's (1966) immunologischem Lebensprofil in der Altersimmunschwäche und ist somit offenbar ein immunologisches Problem an vorgeschädigter Schleimhaut

SUMMARY

On the basis of a grading of the stromal reactions and the histomorphological features of regional lymph nodes in laryngeal cancer, we attempted to test the body s own capacity and sequence of defence as regards immuno biology We distinguish such reaction types as chronic inflammatory epithelial cell tuberculoid and aggressive, *in the stroma of the primary tumour and we compare* them with sinus reactions in regional lymph nodes The latter seem to represent the peak of the body s own ty since they are scarcely ever observed in cases of diable cancer The morphological findings permit n prognostic conclusions patients with aggressive omal reaction and typical sinus reaction in lymph *nodes survive 5 years or more after operative treatment*

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DISCUSSION

P M Kluyskens Did you find any correlation between D N C B test and the stroma reaction around tumour and in the lymph nodes?

J Kirchner How do we know that the congregation of plasma cells and *lymphocytes at the tumour host inter* face is not simply a response to infection in the cancer rather than an immune response? Do you have long term follow up studies to determine whether the various changes you have shown in the lymph nodes have prognostic value?

L Surjan I have also similar observations but there *are a lot of unsolved problems* It is well known that there are different kinds of lymphocytes with different origin and with different functions Did you make some differentiation among them e g with an immunomorphological method? It would be very important to see the study We more our

It may be supposed that there are different activity to the different directions in the increasing of tumour Further is the type and grade *of the stroma reaction and the* lymphocyte infiltration similar around the whole tumour or otherwise?

The cellular and humoral immuno-reactions against the tumour are very important and it seems *as if the* immuno-therapy will be of good help besides the other therapeutical methods in the near future

J Conley Are there known specific immunological features in these morphological observations? Is surgical

ablation of lymph nodes theoretically contra indicated from an immunological point of view?

G Zechner (Reply) Thank you all for the discussion about the prognosis I may say that all patients with a good sinus reaction live more than 5 years postoperatively

To Mr *Kluyskens* The data correlate well with D C G obtained tests

To Mr *Kirchner* Infection is only an additional challenge to the body s own deferce mechanism We proved all in breast cancer with the same results This cancer is scarcely infected

To Mr *Surjan* All findings are localized, never around the whole tumour That would be the solution to make a generalization of aggressive stroma reaction We checked only cytotoxic and cytolytic reactions on single tumour strands

To Mr *Conley* Our morphological findings fit well into the immunological patterns obtained I want to close my talk with an ultimate statement cancer can be cured surgically but cancer is always an immunological problem. In all our efforts we only help the body s own defence we shall never interfere with immunological reactions

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RELATIONSHIP BETWEEN THE VEGETATIVE INNERVATION AND THE SENSIBILITY OF THE NASAL MUCOSA

Z Krajina and Ž Poljak

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Abstract Experimentally and clinically, we observed that the parasympathicomimetic reaction of the nasal mucosa was congruent with higher sensitivity of the nasal mucosa The opposite is true of the sympathicomimetic reaction The reason for this difference in reaction is connected with the transmitting agent of the vegetative system This is shown in the provocation of intradermal reaction of different allergens by these transmitting agents

In 1955 Goodman & Gilman stated that vegetative denervation causes a cell to become more sensitive to chemical mediators and to other non-specific irritations Various opinions exist as to the cause of the increased sensibility fter the denervation of the cell, but the greatest ortance is attributed to the disturbed or the ased permeability of the cell We were very rested to see which part of the vegetative innervation of the nasal mucosa had the greater influence on this hypersensitivity

Autonomic innervation of nasal mucosa

Parasympathetic supply of the nasal mucosa is dominated by the vidian nerve *vis-a vis* the greater superficial petrosal nerve, which latter has the synapse in the sphenopalatine ganglion The other, smaller part leads to the maxillary and ethmoid nerves Sneezing reflex and vasodilatation are the most important symptoms of parasympathetic stimulation No sneezing efforts are made when nasal branches of the maxillary nerve are stimulated Instead, complex facial twitching, jaw clenching and withdrawing movements occur Stimulation of the ethmoid nerve produces vasoconstriction as the principal response, but vasodilatation has been demonstrated and feeble sneezing efforts also occur However, it appears that most of the cholinergic fibres are in the vidian nerve which innervates the most active areas of the nasal mucosa of the lower and the middle turbinate as well as the corresponding part of the septal mucosa The cholinergic nerve fibers terminate mainly in the nasal glands, the arteries and the arterioles and, to lesser extent, in the sinusoids There were no cholinergic fibers directed to or terminating at the ciliated epithelium including the goblet cells

The sympathetic fibers to the nose arise, like the fibers to other parts of the head, from the upper thoracic segments of the cord and perhaps from the eighth cervical segment, these preganglionic fibres ascend to reach the superior cervical ganglion in which they synapse Postganglionic fibres from the superior cervical ganglion pass along the internal carotid artery as the chief component of the internal carotid plexus and those destinated for the nose leave the plexus as the deep petrosal nerve, which joins the greater petrosal to form the vidian nerve, the sympathetic fibers pass through the pterygopalatine ganglion without synapse to be distributed with its branches of the external and internal arteries into the nose give also the sympathetic supply to the nasal mucosa

The vascular nasal system is richly provided with sympathetic fibers, while the nasal glands are almost devoid of adrenergic nerve fibres and then are only occasionally seen near the glands In this way the cholinergic innervation is predominant in the functioning of the nasal glands, while adrenergic innervation is predominant in

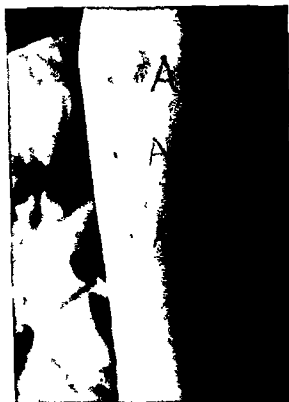


Fig 1 Intradermal reactions with acetylcholine allergen, noradrenalin allergen and allergen, in non-allergic patient

the functioning of the vascular system of the nasal mucosa Of all the organs of the body, the nose is the most sensitive to adrenaline—five times more sensitive than the heart

MATERIAL AND METHODS

In 1961 Krajina & Poljak experimentally caused the interruption of the sympathetic supply to the nasal mucosa in rabbits in order to be able to ascertain in which way such a mucosa reacts to the mechanical physical and chemical irritation To produce a complete sympathetic denervation, denervation of the carotid was regularly done in addition to the removal of the cervical sympathetic ganglion They found these reactions were much more pronounced on the side of the nasal mucosa denervated by the sympathetic supply

In 1972 Krajina et al repeated the same experiment, with dogs They found a stronger sensibility of the nasal mucosa to cold, registered with EMG on the intercostal muscles They concluded that the parasympathicomimetic reaction of the nasal mucosa leads to the stronger sensibility of the nasal mucosa which is reflected not only on the nasal mucosa but also on the lower respiratory tracts

The effect of the exclusion of the parasympathetic nerve and the sensibility of the nasal mucosa has been studied in cases of vasomotor rhinitis which had been subjected to vidian neurectomy In 20 cases, vidian neurectomy was done unilaterally During the operation and 10 days subsequently, the nasal mucosa was taken for histological examination in order to observe the effect of vidian neurectomy In all our histological studies we have found a very pronounced histological difference between the nasal mucosa before and after vidian neurectomy While the mucous membrane of the nose before the cutting of the nerve showed very numerous mucosa glands in the hyperactive state, the glands decreased in number after the cutting, in the stage of normal and stabilized secretion Moreover, eosinophils in the mucous membrane of the nose disappeared after vidian neurectomy

As the acetylcholine is the principal mediator of parasympathetic postganglionic fibres and noradrenalin of the sympathetic postganglionic fibres we wanted to see the effect of these mediators in the provocation of the intradermal reaction in both non allergic and allergic patients The allergen was diluted with the acetylcholine and noradrenalin and the effects of these cutaneous reactions were compared While the acetylcholine allergen provoked much more pronounced cutaneous reaction in non allergic and allergic patients, the noradrenalin-allergen had very feeble or no reactions (Figs 1, 2)

DISCUSSION

The autonomic innervation of about three-quarters of the nasal mucous membrane reaches the nose via the vidian nerve and follows the distribution of the second division

geminal nerve to the nose The destination is the same but the terms vegetative and somatosensory qualify their separate purposes Parasympathicomimetic reaction of the nasal mucosa leads to its stronger sensibility which is reflected not only on the nasal mucosa but also on the lower respiratory tracts In contrast to the prevalence of the sympathetic influence, this hyperreactivity of the nasal mucosa is abolished

This divergency between the sympathetic and parasympathetic system is found on the effects of mediators of both systems on the nasal mucosa

The main parasympathetic ganglion of the nose, the sphenopalatine, is exposed to different external stimuli and, on this way, to cause the parasympathicomimetic reaction, this divergence can be the result of adrenergic insufficiency Szentivanyi (1968) has shown that adrenergic receptors do not respond if there is an inadequate supply of glucocorticoids The latter are capable of sensitizing the adrenergic target cells to the action of catecholamines In atopic disorders a failure of the adrenergic homeostatic mechanisms which normally counterbalance cholinergic stimuli may be the chief effect



Fig 2 Intradermal reactions with acetylcholine-allergen, noradrenalin allergen and allergen in allergic patient

RÉSUMÉ

La reaction parasympathicomimetique de la muqueuse nasale est en correlation avec la sensibilité augmentee de la muqueuse nasale comme nous l'avons constaté par des expériments et en clinique La reaction sympathicomimetique a des conséquences contraires La raison pour la différence entre ces deux réactions est en connexion avec l'agent transmetteur du système végetatif Nous le prouvons en provoquant une réaction entrecutanee par des allergenes différentes ensemble avec cet agent transmetteur

ZUSAMMENFASSUNG

Experimentell und klinisch bemerkten wir dass die parasympathikomimetische Reaktion der Nasenschleimhaut in Zusammenhang mit grosserer Nasenschleimhautempfindlichkeit ist Mit der sympathikomimetischer Reaktion ist es aber umgekehrt Der Grund solch verschiedener Reaktionen steht in Beziehung mit dem übertragenden Agens des vegetativen Systems Dies wird durch Provozieren intradermaler Reaktion mittels verschiedene Allergene samt ubertragenden Agensen bewiesen

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DISCUSSION

H. Naumann: How long does the effect of a surgical interruption of the Vidian nerve last in man and in animal, i.e. how many weeks or months, on average? Do you believe that there is a *direct* influence of cutting the Vidian nerve on the immuno-specific cells like T-cells, B-cells, plasma cells, eosinophils and mast cells?

A. El Mofty: Have you looked for the mast cells in your experiment, as regards their number and their state of granulation? I published a paper 5 years ago about the changes in the nasal mucosa of the Vidian nerve section where the mast cells increased in number and in granularity. In another work on dogs, sympathectomy caused a state similar to atrophic rhinitis in man, where the mast cells increased in number and state of granules tremendously while the contrary happened after parasympathectomy.

Z. Krajina (Reply) to Mr *Naumann*: Our observations on the animals were for a month, and on patients, for the past 10 years. One patient got a relapse of vasomotor rhinitis 2 years after Vidian neurectomy but on the side opposite to the operation. About the influence of the

tomine and noradrenalin.

To Mr *El Mofty*: We did not look at the mast cells in this work.

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NASAL MUCOSA IN THE IRON DEFICIENT STATE

A Clinical and Electron-microscopic Study

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Abstract Good results of iron therapy obtained in ozena patients with significantly lowered serum iron level encouraged studies on the pathogenesis of the disease In electron microscopic examinations special attention was paid to the considerable disintegration of connective tissue stroma in the mucosa In some connective-tissue cells ferruginous micelles were found in the mitochondrial matrix Mitochondrial deposits may be a morphological expression of disturbed iron metabolism at subcellular levels

Clinical experiments in treating ozena with iron compounds, based on a material both sufficiently large (70 patients) and representative of this disease, allowed us to corroborate the correctness of Bernát's opinon (1960, 1965) as to its etiopathogenesis

A high percentage of very good (48 %) and good (27 %) results of treatment (1974) prompted us to undertake more penetrating investigations which might shed light on the mechanism of the therapeutic results obtained

In 3 patients, members of the same family (mother aged 33, son aged 13 and daughter aged 6), suffering from ozena with signs of iron deficiency, electron microscopic examinations of nasal mucosa were performed before and after 9 months of treatment with iron compounds

We paid attention to the fact that the main pathologic changes on the ultrastructural level in the nasal mucosa of these patients were mainly present in the stroma of connective tissue

We found a disintegration of the basal lamina of metaplastic multilayer squamous epithelium (Fig 1) and a general derangement of connective tissue of the stroma (Fig 2) In this stroma we also observed changes in the walls of minute arterioles (Fig 3)

In one case were found tubular paramyxovirus like inclusions in intercellular spaces of the connective tissue (Fig 4)

Those observations, though indicative enough of the stroma of mucosa as the original and principal site of pathologic changes in chronic atrophic rhinitis, did not account for the pathogenesis of those changes

Our latest observations seem to indicate that the pathogenesis of atrophic rhinitis of nasal mucosa is associated with disturbances in the metabolism of connective tissue

In nasal mucosa of the patients with evident iron deficiency we have often observed changes in the mitochondria of connective tissue cells Most frequently the changes consisted in a considerable swelling of the mitochondria, with focal rarefaction of matrix and formation of abnormal cristae (Fig 5)

Relatively often were also encountered mitochondria containing numerous granules of very high electron density (Fig 6)

The granules were always found in the matrix of mitochondria between the cristae In some mitochondria they formed large aggregates (Fig 7)

On comparison with data from literature (Bessis & Breton Gorius, 1957, Bessis & Jensen, 1965, Bessis, 1973) the granules of high electron





Fig. 1. The basal part of the epithelium. The basal lamina (*bl*) shows discontinuity (*arrows*). The arrangement of the epithelial cells is loosened (*ep*). The cells penetrate into the connective tissue (*ct*) of the stroma.

Fig. 2. Connective tissue (*ct*) beneath the epithelium (*ep*). Collagen fibres are disrupted, irregularly scattered and mixed up with fragments of disintegrated cells. Basal lamina (*bl*) of the epithelium.

Fig. 3. The blood vessel of nasal mucosa. The lumen of the vessel has collapsed and is confined by a thin layer of degenerating endothelial cells (*end*). On the outside of the endothelium a thick undulating layer of homogeneous substance is visible (disintegrated basal lamina).

Fig. 4. Microtubular paramyxovirus-like inclusions in intercellular space (longitudinal sections: big arrow; transverse sections: small arrow).

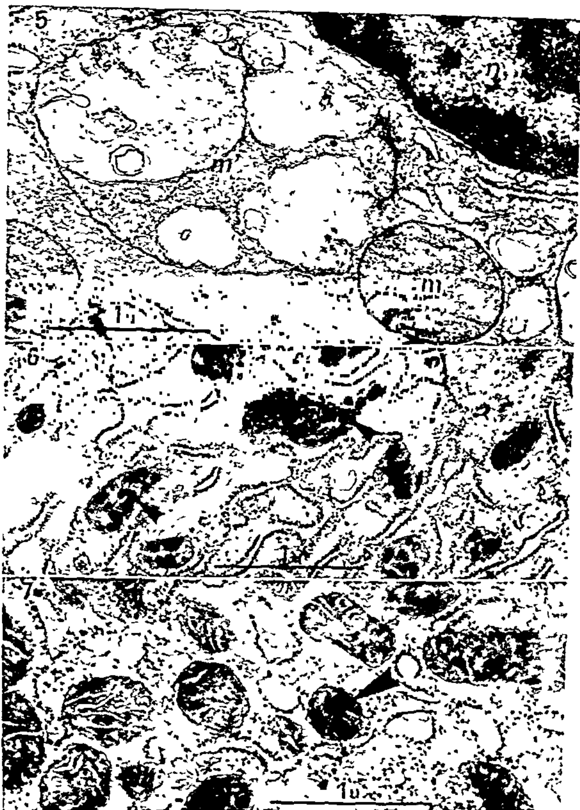


Fig 5 Fragment of connective tissue cell of the stroma of nasal mucosa. Close to the nucleus (*n*) a normal mitochondrium (*m*) and giant mitochondrium (*m*) with focal rarefaction of the matrix and formation of abnormal cristae

Figs 6, 7 Fragments of cytoplasm of connective tissue cells. Mitochondria contain numerous granules of high electron density (*arrows*)

density in the mitochondria of connective tissue cells were found to be identical with those previously observed by other authors in mitochondria of the cells of erythroblastic series, in patients suffering from sideroblastic hypochromic anemia, thalassemia or acute leukemia

Those granules, called ferruginous micelles by some authors, are particles measuring 7–30 Å and probably correspond to the micelles contained in the particles of ferritin Their presence in mitochondria may be indicative of a disturbed utilization of iron particles in a distorted metabolism of the cell They may produce a toxic effect in mitochondria, which, in turn, necessarily leads to serious disturbances in respiration of the cell

We would like to state in conclusion that, in our opinion, the pathogenesis of a chronic atrophic rhinitis of nasal mucosa consists in an original complex disorder of the connective tissue metabolism The clinical and ultrastructural observations seem to indicate that one of the links in the disturbed process may be a distorted iron metabolism in connective tissue cells It is difficult to say at present to what extent these disturbances affect many tissues or if they are limited to nasal mucosa alone

We hope that further investigations will shed more light on the obscure passages

ZUSAMMENFASSUNG

Bei Kranken mit atrophischen Veranderungen in der Nasenschleimhaut wurde ein deutlicher Eisenmangel festgestellt Sehr bemerkenswert war in dem elektronen mikroskopischen Bild die erhebliche Desintegration der bindegewebllchen Schleimhautstroma In manchen Bindegewebezellen fand man in der mitochondrialen Matrix eisenenthaltende Mizellen Diese mitochondrialen Ablagerungen konnen als morphologischer Exponent von Storungen des Eisenmetabolismus auf dem subzellularen Niveau betrachtet werden

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DISCUSSION

J Kirchner You have raised the interesting question as to whether low tissue iron might interfere with cellular respiration If so this could represent a pre malignant condition Low tissue iron is found in Plummer–Vinson or Paterson Brown Kelly syndrome and we know that cancer of the hypopharynx occurs in this condition Do you therefore think that the changes you have demonstrated may lead to cancer?

M S Karatay Were all patients including iron deficiency cases treated for ozena? If they were studied clinically, what changes were noted in their blood films? Because lately we had 88 patients with iron deficiency studied by three colleagues in our clinic, and who showed no changes in mucosa, though we found underdeveloped maxillary sinuses due to hypotrophic and hypoplastic changes in the maxillary bone marrow

Z Bochenek Did you find granules in the mitochondria also in the other cases of your series of patients with chronic atrophic rhinitis?

M Arslan An auto-immune mechanism could be a pathogenic factor of atrophic rhinitis as this disease is a typical example of chronic alteration of the connective tissue

A Zakrzewski (Reply) to Mr *Kirchner* The answer is yes We studied ozena as a model of disorder within the mucosa, which can lead to precancerous and even cancerous state In China ozena is as frequent as cancer of rhinopharynx

To Mr *Karatay* Not all our cases of ozena have the same degree of iron deficiency

To Mr *Bochenek* We have studied only 3 patients—of the same family—and the more spectacular changes in the stroma of nasal mucosa were found in one patient, the most resistant to iron therapy

To Mr *Arslan* You are right The microtubular structures (paramyxovirus like inclusions) found by us in one case only may prove that ozena as well as lupus erythematosus and other immunological diseases may have a similar pathological basis

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ULTRASTRUCTURAL STUDY OF THE HUMAN UTRICULAR MACULA AND VESTIBULAR NERVE IN MENIÈRE'S DISEASE

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Abstract The authors have carried out an ultrastructural study of the human utricular macula and the vestibular nerve in the internal acoustic meatus of four patients suffering from Menière's disease They confirm the presence of degenerative alterations in the utricular sensory epithelium The nervous fibers situated in the supporting connective tissue of the neuro-epithelium showed modifications in the Schwann cell cytoplasm and in the arrangement of the myelin sheaths The same alterations appear in the vestibular nerve in the internal acoustic meatus in 2 of our cases In the other 2, the vestibular nerve was formed by a granular matrix with several myelin figures in the proximity of possible Schwann cell nucleus debris

Meniere's disease still remains a pathogenetic enigma We have endeavoured to confirm ultrastructural findings previous to ours, made by Pietrantoni & Iurato (1960), Litton & Lawrence (1961), Ireland & Farkashidy (1963), Friedmann et al (1963, 1965), Hilding & House (1964), Spoendlin (1970), and Friedmann (1967–1974)

We have studied the vestibular sensory epithelium plus a sectioned vestibular nerve in the internal acoustic meatus

MATERIAL AND METHODS

Our studies were based upon a clinical diagnosis of Meniere's disease in 4 patients Three were males, 25, 40 and 55 years of age and one was a 30-year-old female The first 2 patients had a 2-year clinical history of Meniere's disease, while the latter 2 had a 4- and 6-year history, respectively

In the first clinical case, audiometric studies showed a normal hearing on the left side, but an 80 dB sensorineural loss on the right There was a 100% SISI score and a type II Bekesy The Hallpike caloric test with electronystagmographic recording confirmed a nystagmus preponderance to the right

In the second case the audiometric evaluation showed a profound deafness of the left ear and a normal right one The Hallpike caloric test gave a preponderance to the right with a caloric inhibition at 44°C In both cases a translabyrinthine section of the vestibular nerve was performed Upon exposure of the external and superior semicircular canal a haemorrhagic aspect of the membranous labyrinth was observed

Cases three and four presented a sensorineural hearing loss and a caloric paresis of the affected labyrinth A transtemporal neurectomy was performed on the vestibular nerve

X-ray studies of the internal acoustic meatus were normal in all cases All 4 patients received medical treatment previous to the operation

During surgery the specimens were immediately fixed in a 2% solution of glutaraldehyde in phosphate buffer at pH 7 4 Details of our technique has been published in previous papers (Marco et al, 1971 and Sanchez-Fernandez et al, 1972)

FINDINGS

The specimens having a better state of conservation were those whose epithelium was folded upon itself Initially we will describe those cells whose morphology are in a better state of

conservation and posteriorly those that have major alterations

Sensory cells

The cuticular portion The sensory cell surface diameter is approximately 3 μm The cuticle thickness is normally about 0 5–1 μm The junctional complex between the sensory and supporting cells are preserved, being formed by a zonula occludens, a zonula adherens and a macula adherens At this level a solid union is established between the cuticular surfaces of both cells which appear reinforced and present a darker zone The cuticular plate is formed by a finely granulated material where at times one can distinguish stereocilia rootlets The cell surface is lacking in cilia In only a few cells is it possible to observe small protrusions on the implantation point of the stereocilia roots (Fig 1*a*)

The infracuticular portion contains a group of altered mitochondria, lipid droplets, lipofuscin pigment granules, polyribosomes, smooth and rough-surfaced endoplasmic reticulum that contains a dispersed fine granular material The Golgi complex and microtubules can be situated in its vicinity (Fig 1*b*)

The supranuclear region contains numerous lipid droplets and the endoplasmic reticulum cisternae which appear normal In the *nucleus* the enlargement of the perinuclear space is a constant finding in the sensory and supporting cells

Infranuclear region It is occupied by numerous dilatations of the endoplasmic reticulum, Golgi complex and a few altered mitochondria

Intra epithelial nerves Some conserve their normal morphology The same can be said of several nervous endings (Fig 1*c*)

Supporting cells

The surface plasma membrane is devoid of microvilli In well preserved specimens the cellular junctions are established by a complex union and desmosome reinforcement at the cuticular level

The reticular membrane consists of a granular material and offers fenestrae through which a connection exists between the supra and infracuticular cytoplasmic areas of the cell At this level one observes multivesicular bodies, lipid droplets, dilated endoplasmic reticulum cisternae and lipofuscin pigment granules (Fig 1*d*) The infranuclear region only has a dilated endoplasmic reticulum cisternae (Fig 2*a*)

The zones of the specimen that are very altered show a rupture of the plasma membrane which extrudes all the cell components into the endolymphatic space (Fig 2*b*) In other areas the opening of the plasma membrane extrudes the endoplasmic reticulum contents (Fig 2*c*) Needle-like structures may be observed in the endolymphatic spaces and in the cellular cytoplasmic area of degenerated cells near the lipofuscin pigments granules (Fig 2*d*)

In the endolymphatic space, one can detect numerous cellular debris corresponding to sensory, supporting, or red blood cells

Supporting connective tissue

We describe nervous fiber alterations and then the connective tissue modifications

Nerve fiber In the myelin sheath one of the first alterations may be the presence of split lamellae which appear in the intermediate line and may be accompanied by fusion of several lamellae in close proximity Lamellae splitting also occurs near areas in which the myelin lamellae are completely normal In other zones there is a complete fusion of all lamellae and the myelin sheath appears as an amorphous black area In these modifications the axon remains in a normal state (Fig 3*a*)

The splitting of the innermost myelin lamellae and the appearance of myelin figures plus spherical bodies that are present within them, probably represent metabolic end products of the altered myelin sheaths In the cytoplasm of the Schwann cell, one may observe spherical, oval or rectangular bodies whose major diameter may be 1 5 to 2 5 μm They are normally limited by a membrane which in certain areas

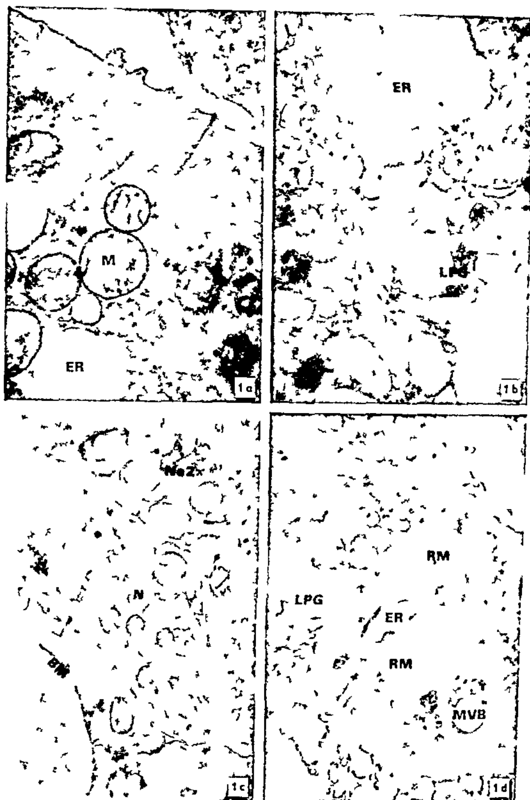


Fig 1 (*a*) Cu ular port on of a sensory cell Note the stereocil a rootlets, the m tochondria (*M*) and some d lated endoplasm c ret culum cis ernae (*ER*) 36 000 (*b*) Infracu cular reg on of a sensory cell Observe the l pofuscin p gment granules (*LPG*) and the d la ed cisternae of the smooth and rough endoplasmic ret culum (*ER*) 30 000 (*c*) Intra-ep the al nerve (*A*) Nerve end ng typ 2 (*Ne 2*) No e the neurotubules and the m ochondria 24 000 (*d*) Support ng ell Re cular membrane (*RM*)) Mul ves cular body (*MVB*) ER and LPG as n the preced ng f gures 24 000

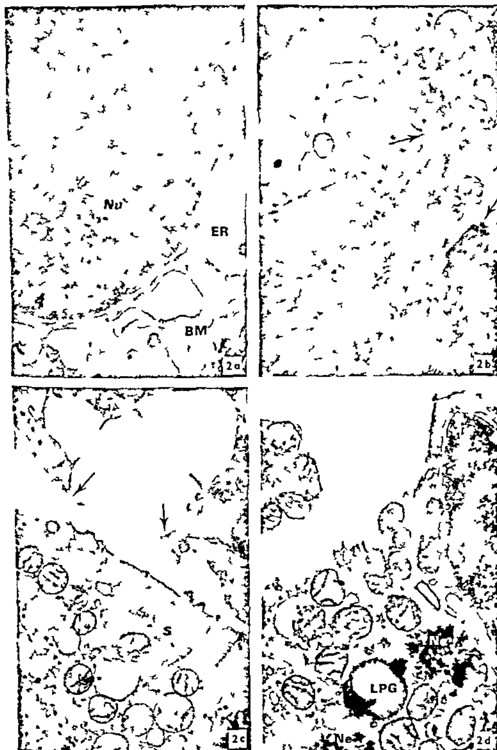


Fig 2 (*a*) Suppo t ng cell Infranu lear reg on Nucleus (*Nu*) Basement membrane (*BM*) 15 000 (*b*) Rupture of the plasma memb ane (*arro*) through wh ch one may observe the extrus on of a ous m tochondr a and part of the nucleus ?4 000 (In h s sect on the ep thel um s folded upon self Sensory cell (*S*) No e the open ng (*arrows*) of the endoplasm c ret culum c sternae of a support ng cell n th endolymphat c space ×24 C00 (*d*) No e n dle l ke structures (*Ne*) in a cytoplasmic area and in o a m tochondria of a degenerated cell d vo d of plasma m mbrane L pofusc n p gment granules (*LPG*) ×30 000

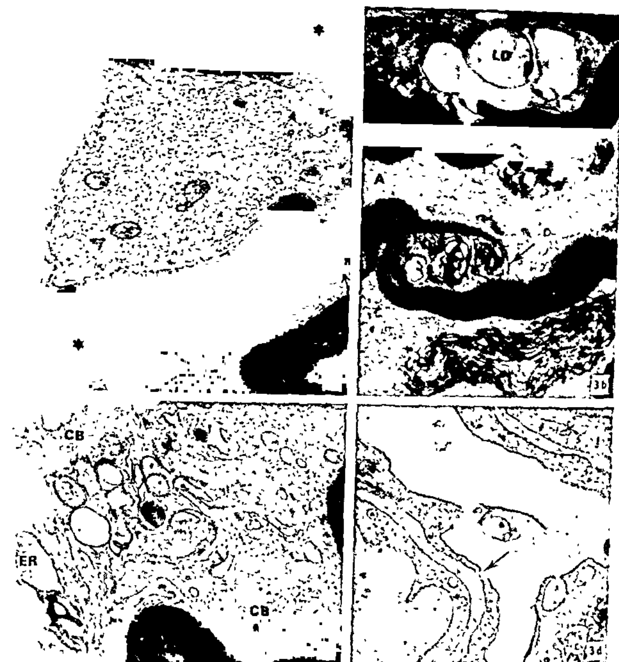


Fig 3. (*a*) Utricular macula Nerve fiber Note lamellae splitting (*arrow*) and the homogeneous areas (*) in the myelin sheath. The axon remains normal For more details see the text. ×30 000 (*b*) Nerve fibers Observe the [illegible] connective tissue. Schwann cell nucleus (*Nu*) In the cytoplasm of the Schwann cell one can observe the dilated perinuclear space and the endoplasmic reticulum cisternae containing a myelin figure Collageneous fiber bundle (*CB*). For more detail see the text. ×24 000 (*d*) Capillary. Note a fenestrae in the endothelial layer (*arrow*) ×36 000

is thick, due to deposits of dark formation of great electronic density upon it. The content of these spherical bodies is homogeneous and may be lipid droplets (Fig 3*b*)

Other myelin sheaths present a marked process of decomposition and are completely disorganized, appearing as a concentric lamellar structure.

It is possible to find lipofuscin pigment granules in the Schwann cell cytoplasm. In these cells the smooth and granular endoplasmic reticulum appears dilated. Occasionally, in its interior, myelin figures appear. A frequent finding is the amplification of the perinuclear space (Fig. 3*c*).

The plasma membrane of many cells breaks out and its components are dispersed to the interior of the connective tissue. The fundamental component of the connective tissue is a finely granular material in which one can distinguish some collagenous fiber bundles and numerous free cytoplasmic components such as lipofuscin pigment granules, endoplasmic reticulum cisternae, lipid droplets and altered mitochondria (Fig. 3*c*).

Some capillaries appear with a normal ultrastructure. In others, it was possible to observe pores in the endothelial layer and oedema alterations in the cytoplasm of pericites and of some endothelial cells (Fig. 3*d*).

Study of Vestibular Nerve in the Internal Acoustic Meatus

In 2 of our cases in which we have already commented about the injury at the sensory neuroepithelium level, the vestibular nerve in the internal acoustic meatus showed alterations of the myelin sheath very similar to those which we have referred too.

The splitting of myelin sheaths at the intermediate line initially concerns the innermost lamellae. Occupying the interior of these spaces are round or ovoid structures. They have a peripherical wrapping, are very electron dense and are formed by numerous dark granules. The axon maintains its normal morphology (Fig. 4*a*). In other areas the splitting affects several lamellae and in the interior of this space a bleb is formed in the myelin sheath. Some of its lamellae appear fused and disposed around some dark granules located in the center of this prolongation (Fig. 4*b*).

Others homogeneous myelin sheaths show a great cleavage of the innermost lamellae, forming an ovoid prolongation into the axon area. In the interior of this prolongation the lamellae splits and myelin figures appear. Also black areas can be seen with numerous black granules of high electron density (Fig. 4*c*).

Transtemporal neurectomy

In the 2 cases in which transtemporal neurectomy was performed, the degenerative alterations were more noticeable as no normal nervous fiber was observed. However, we can see a granular matrix with condensation of myelinic figures in proximity to the possible remains of Schwann's cell nucleus (Fig. 4*d*).

Vestibular ganglion

We did not observe any area where it was possible to recognize neuron ganglion cells corresponding to the vestibular ganglion cells. In the sections that probably correspond to the place where the vestibular ganglion cells should be, we have observed the appearance of diverse myelin nerves alongside various cellular debris and nuclei in a state of pyknosis. We have been able to recognize certain zones in which synaptic endings appear.

The endoneural connective tissue capillaries were normal. In this space it was possible to see between the collagen fibers a neutrophil which extrudes its granules to the interior of the endoneural connective space.

DISCUSSION

The ultrastructural findings due to Meniere's disease previously described by authors in the literature were: (i) Loss of the cilia in the sensory cells. (ii) A thinner cuticular plane. (iii) Cytoplasm having a spongy appearance. Our findings confirm these observations.

We have verified in our specimens that the dilatations affect the endoplasmic reticulum cisternae and the perinuclear space of the sensory and supporting cells. This dilatation frequently occurs with the influx of sodium and water into the cell as pointed out by Trump & Arstila (1971) in cell injury showing an altered steady state. Hilding & House (1964) have also

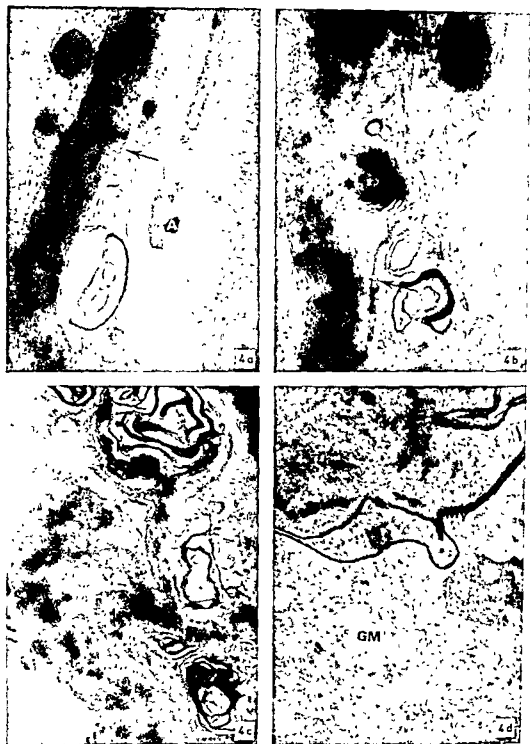


Fig. 4. Vestibular nerve in the internal acoustic meatus. (*a*) Splitting of the innermost lamellae (*arrow*). Axon with a normal morphology. × 36 000. (*b*) Splitting (*arrow*) and myelin sheath bleb (*). × 36 000. (*c*) See the text. × 30 000. (*d*) Granular matrix (*GM*) and myelin figures (*Mf*). × 24 000.

observed a vesicular degeneration in the utricular epithelium of three patients suffering from an acoustic neuroma and another affected with clivus meningioma. Litton & Lawrence (1961) and Ireland & Farkashidy (1963) believe that saline irrigation and surgical handling in vestibular labyrinth extraction during the operation period may provoke vesicular degeneration.

The accumulation of lipids within the cell cytoplasm of sensory and supporting cells has been pointed out by Friedmann et al (1963, 1965) and ourselves and may represent a common sublethal reaction to injury (Trump & Arstila, 1971)

The presence of lipofuscin pigment granules may signify a wear and tear process as indicated by Sehrt (1904), Fawcett (1966), Porta & Hartroft (1969), Bourne (1973) and others

We have been unable to observe the laminate structures that appear in the utricular neuroepithelium cell cytoplasm in Meniere's disease and in a case of Conn's syndrome as described by Friedmann et al (1963, 1965) and Friedmann (1974), as well as by Hilding & House (1964) in specimens from non Meniere s cases as often as in Meniere s cases

In Schwann cells we also observed dilatations of the endoplasmic reticulum cisternae and of the perinuclear space, and the accumulation of lipofuscin pigment granules This has made us consider that these cells may present the same degenerative response to cell injury as sensory and supporting cells

In those specimens having a markedly altered area the interruption in the plasma membrane continuity with cytoplasmic element extrusion express a lethal injury, as they have surpassed the "point of no return" (Trump & Arstila, 1971), and the negative feed back control mechanism is lost

The presence of needle like structures in the endolymphatic space and in the cytoplasmic areas may be interpreted as hydroxyapatite crystals proceeding from the otoconia destruction or from an accumulation of ionic calcium and phosphate of the degenerated mitochondria in cell injury, as demonstrated by Trump & Arstila (1971)

We have been unable to find the collagen like bands (broad banded collagen filament) as described by Ireland & Farkashidy (1963), Hilding & House (1964), Friedmann et al (1965), Friedmann (1967, 1974), and Spoendlin (1970)

Myelin sheath alterations retaining a normal axon morphology may denote a primary myelin sheath disease, as has been previously referred to by Bischoff (1970), Kocen et al (1973) and Joosten et al (1974), in several diseases of the peripheral nerves However, when these alterations affect both, the myelin sheath and the axon, a retrograde degeneration in the vestibular ganglion cell may occur Its departure point may be at the level of the degenerated vestibular neuroepithelium

The same occurs in Corti's organ where a relationship exists between sensory and neural degeneration, as described by Spoendlin & Gaceck (1963), Kerr & Schuknecht (1968), Johnsson & Hawkins (1972), Johnsson (1974) and Spoendlin (1974)

Finally, we speculate that neuroepithelium and vestibular nerve alterations may be evolutionary stages of the same disease We shall continue this investigation in order to confirm this finding

RESUME

Les auteurs ont effectue une étude ultrastructurelle de la macula utriculaire et du nerf vestibulaire au fond du conduit auditif interne chez quatre patients qui souffraient de la maladie de Meniere Ils constataient la presence d alterations dégénérescents dans l épithelium sensoriel utriculaire Les fibres nerveuses situees dans le tissu connectif de soutien de l epithelium neurosensoriel presentaient des modifications des gaines de myeline Dans deux de nos cas les memes alterations aparaissaient dans le nerf vestibulaire au fond du conduit auditif interne Dans les autres deux cas le nerf vestibulaire etait formé par une matrice granulaire avec quelques formations de myeline pres de possibles restes de noyaux de cellules de Schwann

ZUSAMMENFASSUNG

Die Verfasser haben eine ultrastrukturelle Studie des utrikularen Flecks und des vestibularen Nervs im Boden des inneren Gehorgangs bei vier Patienten die eine Menièresche Krankheit hatten vorgenommen Sie beweisen das Vorhandensein von Degenerationsveranderungen im utrikularen sensorichen Epithel Die Nervenstrange die im Verbindungshaltegewebe des neurosensorischen Epithels gelegen sind, zeigten Veränderungen im Zytoplasma der Schwannzellen und in der Verteilung der Myelinscheiden. Dieselben Veranderungen erschienen am vestibularen Nerv im Boden des inneren Gehorgangs bei zweien unserer Falle Bei den beiden

anderen war der vestibulare Nerv aus einer kornigen Gebärmutter mit einigen Myelinformen in der Nähe von eventuellen Kernresten der Schwannzellen gebildet

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DISCUSSION

I Friedmann I have been very interested in this demonstration but I could not help feeling that the degenerative changes described might not all have been due to Menière's disease I would like to ask you whether the interval between operation and fixation might have been too long. The specimen might also have suffered during the operative removal I have seen no evidence of the various, in my opinion, relevant features, we and others have described, e g long-spaced collagen structures. May I mention also the finding of ciliated epithelium in the semicircular canal in a case (patient of Angell James) Similar findings were described by Hoshino and Matsumoto (1974) in a cat

Sánchez-Fernández (Reply) to Mr *Friedmann* First question The specimens were immediately fixed in a 2% solution of glutaraldehyde Second question We have been unable to observe the "laminate structures" which appear in the sensory cell cytoplasm as described by Friedmann et al (1963 65) and by Hilding & House (1964) We have also been unable to find the "broad-banded collagen filament" described by Friedmann

Acta Otolaryngol 79 189–196, 1975

MENIÈRE S DISEASE
ELECTRON MICROSCOPY OF THE VESTIBULAR GANGLION AND END ORGANS AFTER ULTRASOUND

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Abstract Material for electron microscopy study was obtained from a patient previously suffering from Meniere s disease and treated by ultrasound It consisted of parts of the membranous labyrinth as well as the vestibular ganglion The neuro-epithelium of the macula showed degenerative changes and there was marked vacuolation of the cytoplasm The endothelial cells lining the semi circular canal showed gross vacuoles contained de generating mitochondria and some debris which may reach the endolymph The wall of the semi circular canal was oedematous and the basement membrane lifted off by oedematous fluid There was obvious change in nerves and neurones The nerves showed loosening of the myelin sheath and ballooning The neurones contained lipoid inclusions and multicystic lysosomes Similar inclusions were also noted in some myelinated nerves

The effects of ultrasound on the normal mammalian ear have been well demonstrated in experimental animals by workers such as Angell James et al (1960) and Brain et al (1960) Severe degenerative changes may be produced in the neuro-epithelial structures of the membranous labyrinth and there is general agreement that this is largely due to a thermal effect, although cavitation, altered pH, and enzyme changes have also been noted

However, little material has become available from human patients with Meniere's disease who have been treated by ultrasound Newlands (1966) reported on material obtained from the lateral semicircular canal of a patient who had been treated by ultrasound 2 years previously Surgical destruction of the labyrinth was carried out as the patient had a recurrence of symptoms Newlands found that the lateral semicircular canal was obliterated by fibrous tissue and some endosteal new bone formation was present He was not able to find any recognizable remnants of vestibular end organs

Further observations were made by Friedmann et al in 1972, using electron microscopy Again, labyrinthectomy was carried out for a recurrence of symptoms following ultrasound treatment They reported changes in the neuro-epithelium of the crista and the presence of some cells with microcilia in the membranous canal

We are now reporting the electron microscopy findings in material obtained from the following patient

Mrs E H (Hospital Number 97710) This lady was first seen in 1963 when aged 55 years, with a characteristic history of left sided Meniere's disease Medical treatment was prescribed without obvious benefit and therefore ultrasonic application was carried out to the left labyrinth in June 1965 The operation was uneventful the maximum intensity of ultrasound was 22 watts per cm² The application was considered to be of average duration, i e approximately half an hour

The patient remained fairly well, although hearing tended to fluctuate In 1972 the patient complained of feeling of unsteadiness with the development of a permanent and severe hearing loss Electronystagmography and caloric stimulation suggested the likelihood of some residual labyrinthine function and because of the patient's increasing disability it was decided in October 1972, to carry out translabyrinthine vestibular neurectomy (B H C) The membranous canal



Fig 1 Cuticle of degenerated hair cell with laminated structure

and the ampulla were extracted intact from the lateral semi circular canal, and together with the nerve and ganglion were sent for electron microscopic examination (I F) Relief of symptoms since neurectomy has been dramatic and complete

The electron microscopic appearances are as follows

MATERIAL FOR ELECTRON MICROSCOPY

Embedded material from the following tissues was received and sectioned on an LKB or Sorvall Bloom ultratome and examined under Philips 300 electronmicroscope

Macula of the utricle There was extensiv degeneration of the epithelium, the cytoplasr was vaculated, and there was considerable mitc chondrial damage The cuticular surface wa vesiculated and there were numerous bullou protrusions of the cell surface Near the cuticl laminated structures as previously describe could be seen

The semicircular canal appeared to be vacuo lated in thin sections stained with Toluidine Blue This was confirmed electron microscopically Most of the endothelial cells of the membranou canal show large vacuoles pushing the cel

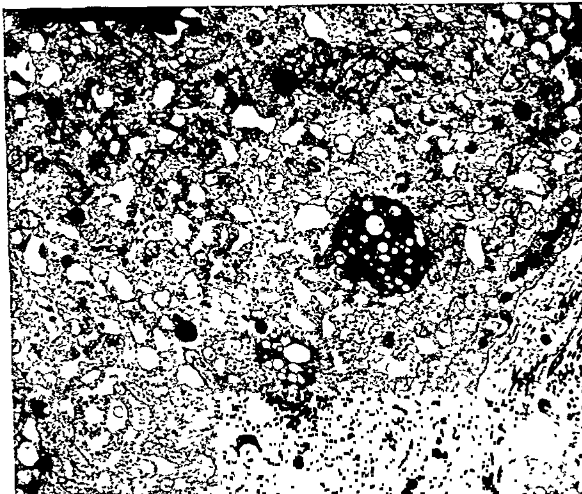


Fig 2 Detail of neurone of vestibular ganglion with distended cisternae of the Nissl substance and lysosomal inclusions

nuclei aside Some of the vacuoles contained some amorphous debris, others contained collagenous fibres indicative of repair Several of the vacuoles were protruding into the lumen and some of these appeared to have ruptured The stroma was oedematous and the basal lamina lifted off the endothelial lining

Neurones of the vestibular ganglion were present in large numbers They showed marked dilatation of the endoplasmic cisternae forming the Nissl granules The ribosomes lining them appeared to be released into the cytoplasm which contained different types of inclusions There were large multivesicular membraneous structures of pale colouration and also darkly stained multicystic lipofuscin granules

The nuclei were hyperchromatic and there were no or very few myelin layers on the cell surface

Myelinated nerve fibres were numerous and showed balooning of the myelin sheath Several contained similar multivesiculated inclusions as seen in the neurones These were often large and had protruded from the myelinated nerve

DISCUSSION

Several of these observations are similar to those described in previous papers (Friedmann

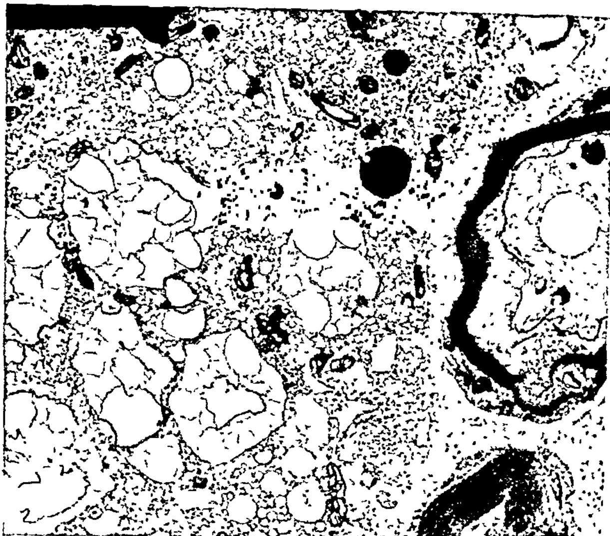


Fig. 3. Detail of neurone of vestibular ganglion containing multivesiculated cytosomes.

et al., 1965*a*, *b*) and by others. Degeneration of the neuro-epithelium was first described by Pietrantoni & Iurato. Laminated inclusions were noted by Ireland & Farkashidy and confirmed by our work. Long spacing fibrous collagen has been noted in other tissues also under pathological conditions (Banfield et al., 1973). This does not detract from the significance of their presence in the human ear in conditions associated with endolymphatic hydrops. It may be that they serve certain specialized functions such as adhesion between the basal lamina of nerves cells and surrounding collagen and become hyperplastic under stress. Rowlatt (1969) has supported the suggestion first made by Palade & Farquhar (1965) that the fibrils and similar structures which he called "possible tropocollagen aggregates" represented attachment points under epithelial cells subjected to stress.

Lack of knowledge of function need not preclude a consideration of their significance in the human labyrinth on exposure to increased labyrinthine pressure.

Vacuolation may have been caused by the cavitation effect of ultrasound. It is interesting to note that Lindemann (1969) has confirmed Werner's (1933) finding of intra-epithelial spaces in the striola of the normal macula utriculi and he speculated that these spaces contained



Fig 4 Myelinated nerve with multicystic inclusion

some special liquid different from both endo and perilymph Watanuki & Kagayama (1972) have described fluid spaces in the central region of the crista ampullaris in the axon or in the chalice of thick nerve fibres in the neuro-epithelium which have lost their myelin sheath

Unusual ciliated cells have been observed by Friedmann et al (1972) in the semicircular canal of a patient operated on for Meniere s disease Subsequently Hoshino & Matsumoto (1974) found a group of ciliated cells in the area of the utricular macula of the cat

The function if any, of these ciliated cells is obscure It is perhaps interesting to note that ciliated cells have been described in the renal tubules in the human fœtus and under pathological conditions such as hypercalcaemia and acute or subacute inflammatory conditions affecting the kidney, including acute and subacute glomerulonephritis, lupus erythematosus, acute renal failure, probably in serum sickness (Wesson & Duckett, 1973) Wesson & Duckett suggested that if such cells rather than representing random embryological arrests of reversions function as physical or chemical receptors then the mechanism of communication to appropriate effectors is wholly unknown Given an efficient mechanism of communication from ciliated cell to these renal elements few receptors would be required

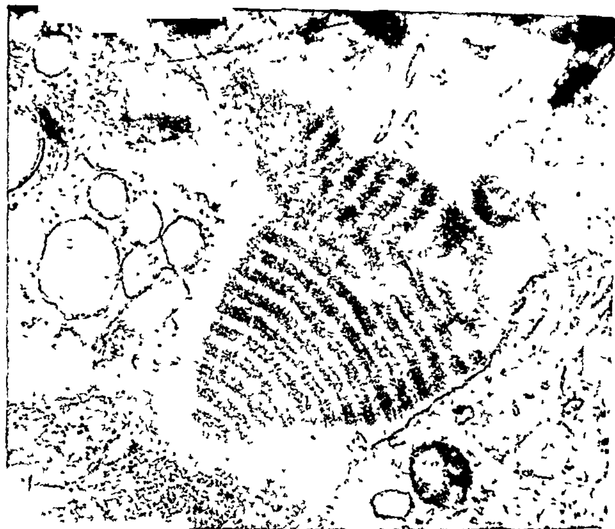


Fig 5 Laminated structure in the stroma of the macula of the utricle formed by fibrous long spaced collagen

These suggestions may perhaps apply also to the vestibular apparatus but must of course await further similar observations

The inclusions in the neurones are of two types and may be of lysosomal character Similarly the lipofuscin granules have been considered by Brunk et al (1972) in ageing neurones to contain lysosomal enzyme and to result mainly from cellular autophagy These structures accumulate intracellularly indicating that the cell is unable to rid itself of some residues by way of exocytosis the cells may have lost their capacity for defaecation as was put rather inelegantly by the discoverer of the lysosome himself (DeDuve et al , 1969)

Some of the changes e g vacuolation cavitation might be due to ultrasonic irradiation and one might wonder whether the release of the vacuolar contents might not in fact contribute to the patient's subsequent discomfort

The ultrastructure of the normal vestibular ganglion cells was described by Ballantyne & Engstrom (1969) who have distinguished a myelinated and non myelinated type Their illustrations show none of the inclusions and other changes observed in our material although they refer to certain ' osmiophilic particles

ACKNOWLEDGEMENTS

Mr D Cottell prepared the sections for electronmicroscopy Thanks are also due to the National Deaf Ch l



Fig 6 Endothelial cell of sem circular canal Note large vacuole and œdematous stroma

dren s Soc ety for a grant n a d (to I F) for the purchase of d amond kn es and to Mr Leach Cl n cal Photographer Imper al Cancer Researcl Fund Laborator es for the llustrat ons

RÉSUMÉ

La mat ere pour les études electron m croscop ques etait obtenu d une malade qu souffra t auparavant de la malad e de Men ère et qu on ava t tra é par ultra-son Cette matiere se composa t de part es du labyrinthe membraneux aussi b en que le gangl on vest bula re Le neuroep thel um de la macule montra t des changements dégénérat fs et l y ava t de la vacuolat on p ononcee du cytoplasme Les cellules endothel ales du canal semic rcula re montra ent de la vacuolat on gross ere (ou exageree) et les vacuoles contena ent des m tochondries degénerantes et des debris qu peuvent gagner l endolymphe ainsi obstruant son coulement Le mur de canal semicircula re eta t œdemateux et la membrane de base eta t soulevee par le flu de œdemateux Il y ava t un changement prononce dans les lysosomes mult cyst ques On nota t auss de pare lles inclus ons dans quelques uns des nerfs myelines

ZUSAMMENFASSUNG

Material fur elektronenmikroskop sche Stud en wurde von e nem Pa ent erhalten der an der Mén ereschen Krankhe t l t und durch Überschall behandelt wurde Es setzte s ch aus Teilen des membranosen Labyrinths

und des vestibularen Ganglions zusammen Das Neuroepithel der Macula zeigte degenerative Abwandlungen, und es kam eine bestimmte Vakuolenbildung des [illegible]

konnten Die Wand des semizirkularen Kanals war odematos, und die Grundmembran war durch odematose Flüssigkeit abgelost Es zeigten sich deutliche Veränderungen in den Nerven und Neuronen Bei den Nerven sah man ein Losen der Myelinscheide und Ballonierung Die Neuronen enthielten lipoide Knoten und mehrzystische Lysosomen Ähnliche Knoten wurden auch in einigen myelinierten Nerven beobachtet

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MORPHOGENESIS AND ULTRASTRUCTURE OF THE MOUSE EMBRYONIC SALIVARY GLAND IN TISSUE CULTURE

Normal, and Following Exposure to Trypsin

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Abstract The primordial submandibular glands of 12 day-old mouse embryos were studied in tissue culture before and after treatment with trypsin under the electron microscope In vitro differentiation proceeded normally and reached a high level of differentiation Following a soak in trypsin for 15 or 30 minutes considerable changes were noted in the basal lamina and in the mesenchymatous cells There often occurred bizarre bullous protrusions of the cytoplasm through the apparently weakened basal lamina and the mesenchymatous cells were converted into so-called ropalocytes Subsequently the cells regained their normal appearance and the basement lamina was covered by a thick layer of amorphous electron-opaque basement membrane like material It is concluded that the basal lamina (the basement membrane under the light microscope) might be the keystone in the differentiation of an organ and its maintenance in the adult. The development of innervation has also been studied and it was shown that the developing submandibular gland is endowed with large bundles of nerve axons surrounded by Schwann cells lying in the epithelial–mesenchymal region Intra-epithelial nerves were conspicuous and occasional synaptic bars or rings could be seen contributing to the differentiation of the secretory cell

Organogenesis is a complex, multi step process, and the study of this problem at various levels has given rise to a very extensive literature, the analysis of which is outside the province of this paper (Hodges 1969)

Tissue interdependence has long been recognized in the developing embryo in particular from in vivo experiments and transplantation studies The continued differentiation of one group of cells is dependent upon the inductive influences of other proximal groups of cells of different types

An intensive study of these tissue inter relationships has been made possible in recent years by the development of methods based on the dissociation of embryonic organs into the constituent tissues or cells (Moscona, 1952, Grobstein, 1953) A new field of research has developed since the 1950 s in which the inductive relations of different tissues and, more especially, the stromal-epithelial interactions have been analysed at the morphological and, to a more limited extent, at the biochemical levels by in vitro culture methods

Tissue culture techniques are invaluable for the study of biological processes at the cellular level In previous studies the ultrastructure and the development of innervation of the chick embryo otocyst in vitro has been studied (Friedmann, 1956 and 1974) It has been shown that the neuro-epithelium reaches full differentiation at the ultrastructural level and that innervation takes place in the centrifugal manner and seems to play a role in the ultimate differentiation of the neuro-epithelial cells

A survey of the literature shows few studies on the ultrastructural development of the embryonic submandibular and sublingual glands in vitro, although observations of the epithelio-mesenchymal tissue junction of the salivary gland rudiment and on the cell surface and microfilament complex of the epithelial salivary cell have been reported (Bernfield & Wessell) 1970) The ultrastructure and cytochemistry of

the acinar cells in the maxillary gland has been studied in the human submandibular gland by Tandler (1962), is adult Sprague-Dawley rats by Vidic (1973), in the European hedgehog by Tandler & MacCallum (1972), in the human parotid gland by Riva & Riva-Testa (1973) and in the adult male mouse by Wrigley (1974, PhD Thesis)

The innervation has been extensively studied by Garrett (1965, 1966 and 1972) Understanding of the developmental capabilities of the embryonic rudiment and of the specific properties of its constituent tissues has been obtained from tissue recombination studies following tissue separation using agents such as trypsin and EDTA (disodium ethylene diaminetetra acetate), (Hodges, 1969) An interesting problem, however, concerns changes in cellular properties caused by treatment with dissociating agents It is important to learn whether surface membranes, the cytoplasmic organelles and the basal lamina are in any way altered

In the present paper our attention has focused firstly, on a study of ultrastructural changes in the epithelial and mesenchymatous cells, (stroma ithelial) of the submandibular salivary gland following trypsin treatment and cul- in vitro, secondly, on the development of vation both in trypsinized and control cultures (as a consequence of the conflicting views in the literature on the innervation of the salivary glands) and thirdly on the role of the "basement membrane" of the light microscopist —the "basal lamina" of the electron microscopist, with some reference to carcinogenesis

MATERIALS AND METHODS

Explants

Pregnant mice of the C57a Icrf strain were killed by neck dislocation, following light carbon dioxide anaesthesia, 12 to 18 days after detection of vaginal plugs The entire uterus was removed *under sterile conditions and placed in a* dissecting dish containing Hanks saline solution The foetuses were decapitated and the lower jaw excised Salivary gland rudiments from the submandibular-sublingual anlage complex were isolated following the basic procedure of Plasmayer (1969) and the stage of development classified according to Borghese's description (1950)

Culture technique

The salivary gland rudiments were cultured on cellophane strips (previously sterilized in 70% ethanol, washed twice in Hanks saline solution over a period of 60 min and equilibrated in culture medium) Each explant assembly was laid over a 3–4 mm hole punched in a stainless steel wire mesh square lying flat on the bottom of optical Anumbra glass petri dishes The amount of medium added (1 5 ml) to each petri dish came to the level of the cellophane substrate The cultures were incubated at 37 C in sealed, humidified Perspex chambers under an atmosphere of 5% CO_2 in air

Culture medium

The culture medium consisted of antibiotic free Waymouth's MB 752/1 medium (Waymouth, 1959) (Wellcome Reagents Ltd, England) supplemented with 0 45 μg/ml ferrous sulphate and with 5% calf serum (Flow Laboratories)

Preparation of trypsin

Bovine pancreatic trypsin (twice-crystallized, salt free, activity 3 000 N F units/mg, Tryptar, Armour Pharmaceutical Co Lot nos RD 2101, TH 1101) was prepared at concentrations of 2 000, 1 000, 100, 10 and 1 μg/ml in Hanks saline solution from stock 1% trypsin solutions stored in aliquots at −20°C The protease activity of bovine trypsin was measured with

Table I *Relationship of trypsin concentration to enzyme activity*

Concentration (μg ml)	Activity (μg ml)
2 000	1 130 9
1 000	521 8
100	55 3
10	6 6
1	2 1

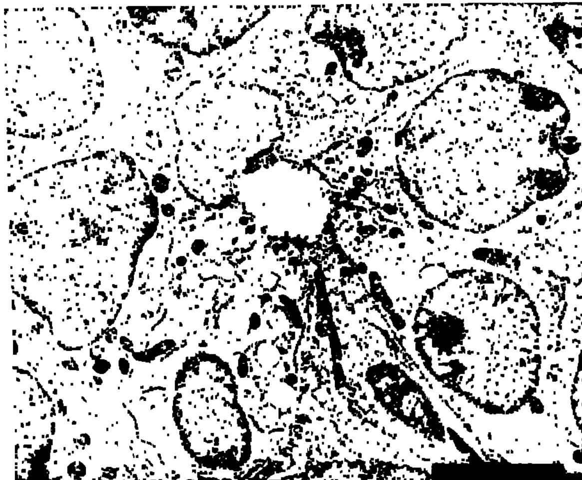


Fig 1 Survey of acinus in untreated culture Note secretory cells with vacuoles and electron-opaque zymogen granules near the lumen ×16 700

N-benzoyl-DL-arginine-*p*-nitroanilide (RAPA) (Boehringer Corp) as substrate (Erlanger et al, 1961) The relationship of trypsin activity to concentration of enzyme employed is tabulated in Table I

Experimental procedures

Only 13 day salivary gland rudiments were used in the in vitro series of experiments Following dissection the rudiments were washed once in Waymouth's ferrous sulphate medium and incubated for 15 or 30 min at 37°C in either 5 ml of Waymouth's ferrous sulphate medium or in 5 ml of trypsin solution preserved at 37°C 15 min prior to use One salivary gland rudiment from each embryo served as a control for its contralateral test partner Following treatment the rudiments were rinsed in 50 50, 10 90 and 5.95 calf serum/Hanks solutions for periods of 5 min prior to culturing

Microscopy

Living cultures were observed and photographed at the beginning of the culture period and at subsequent 24 hr intervals in order to follow the development and growth of the explants

Explants were fixed for light microscopy in Bouin's fluid, embedded in Paraplast, sectioned at 5 μm and stained with haematoxylin eosin

Explants were fixed for electron microscopy



Basal cells Note the th n basal lam na 16 700

at 4 C in 2 5 % glutaraldehyde in 0 1 M sodium cacodylate pH 7 1 for 2 h post fixed in Palade s buffered osmium tetroxide fixative (pH 7 4) for 1 h dehydrated and embedded in Araldite

RESULTS

Trypsin effects on morphogenesis—Macroscopical observations and light microscopy

The development of salivary gland cultures was assessed according to the number of adenomeres in the submandibular anlage produced at d f ferent times in culture

Control salivary glands underwent charac teristic branching morphogenesis in organ cul ture forming multiple lobules with characteris tic acini When the sal vary glands were pre treat ed with 2 000 μg trypsin per mill l tre epithel al morphogenesis was severely curtailed and de generation of the cultures was occasionally ob served Pre-treatment with 1 000 μg or 100 μg trypsin per mill litre also restricted epithel al morphogenesis though the characteristic pat tern of lobulation was ma ntained Measurement of the epithelial areas in explants from the three above treated groups carried out at 24 h inter vals from 0 h to 72 h cultures (when the ep thelial and mesenchymal areas could st ll be easily defined) showed that the ep th el al mass while increasing from time 0 h onwards was always less than that of the control cultures In contrast salivary glands pre treated w th 10 μg trypsin per millilitre showed branch ng wh le



Fig 3 Trypsinized culture (following 30 minutes' soak in trypsin) There are secretory cells surrounding the dilated lumen containing both mucoid and serous secretory granules There is some degenerated matter in the lumen and the cell membrane of one of the cells lining the lumen is protruding into it × 13 600

pre-treatment with 1 μg trypsin per millilitre did not produce changes recognizable at the morphological level There was little evidence of an age-related response of the submandibular anlage to trypsin

Electron microscopy—untreated cultures

The differentiated acini were composed of acinar and basal cells (Fig 1) The acinar cells of the developing submaxillary and sublingual glands were of columnar or pyramidal shape converging on a lumen The luminal surface appeared to be smooth and contained secretory granules There was a variable pattern and some of the cells contained large basophilic zymogen granules surrounded by fairly well defined membranes, whose electron density was not uniform (especially in the trypsin treated cultures)

There were granules made up of partly homogenous dark secretory matter; the remainder of the granules contained some amorphous granular secretory product The zymogen granules were aggregated near the lumen and whole granules were extruded into the lumen without the apparent involvement, of the granular membrane

There were on the whole more secreting cells of the mucus-secreting type clustered around the lumen or canaliculus studded with microvilli They contained electron-lucent granules en-



4 Trypsinized culture (following 15 minutes' soak in trypsin) Note the electron-dense amorphous material filling some of the intercellular spaces and acinus Intact desmosomes are also present Some of the cells contain well formed centrioles × 20 500

closed in membranes The membrane appeared to be less well defined and the granules coalesced to form large, vacuolated structures in these cells The secretory material of these cells reached the lumen through the ruptured plasma membrane of the cells forming the lumen

The original pattern has varied during in vitro development and the final pattern may not have been established when the cultures were fixed for sectioning

Most cells contained a large nucleus in the vacuolated cytoplasm There was, in the perinuclear zone, a well developed Golgi apparatus The basal zone of the cells contained cisternae of the granular endoplasmic reticulum, forming mainly narrow slits which may become dilated

The cytoplasm also contained numerous mitochondria of various size and shape sometimes hidden in the secretory cells, and a rich complement of ribosomes and polyribosomes The cells were closely attached and connected by junctional complexes containing desmosomes with tonofilaments attached Microfilaments and microtubules were sometimes very near the surface, but more commonly found at the base of the cells, running parallel to the basal lamina or alongside the outer cell membrane Centrioles and developing cilia have frequently been observed The basal cells are supporting cells and form a layer of cuboidal or low columnar cells (Fig 2) The controls only showed occasional mitoses contrasting with the enhanced mitotic

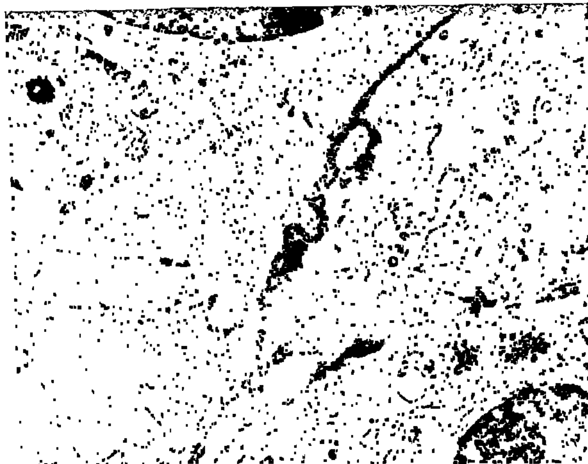


Fig 5 Trypsinized culture (following 30 minutes' soak in trypsin) Note the electron-opaque material along or replacing the basal lamina × 24 200

activity in the trypsinized cultures The basement membrane or basal lamina of the controls was clearly defined and separated the basal cells from the surrounding mesenchymatous cells The mesenchymatous layer was formed by elongated fibroblasts and round cells of histiocytic type (Fig 2)

Electron microscopy trypsin-treated cultures

The effect of trypsin was dependent upon the duration of the preliminary soak

At 15 minutes the differentiation of the acini (Fig 3) has proceeded normally inspite of the marked changes affecting the basal lamina and the mesenchymatous cells

The basal lamina often appeared as a fuzzy line or as a thickened layer of electron-opaque matter much of which has accumulated alongside the basal lamina (Fig 5) and could be seen filling the intercellular spaces The lumen of the acini appeared to be distended and was often filled with variable matter (Fig 4)

There was some increase of the ribosomes and polyribosomes and some mitochondrial change was evident in many cells On the whole, however, the cellular pattern was intact

Mitoses were commonly and easily found, especially in the basal cells and in the mesenchymatous cells (Fig 6) The secretory granules were of irregular shape and size and their contents granular.

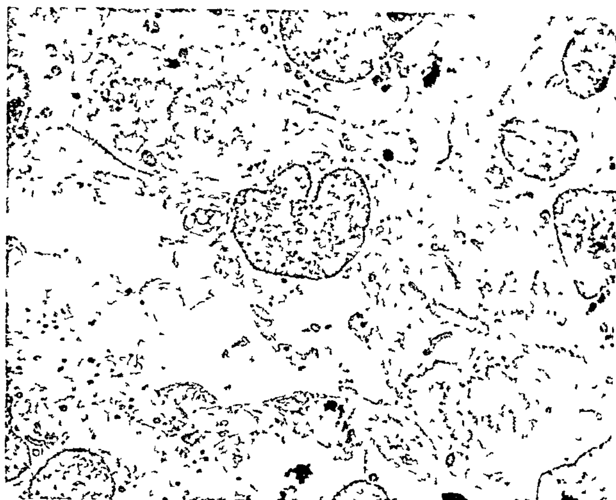


Fig. 9. Trypsinized culture (following 30 minutes soak in trypsin). Mitotic basal and mesenchymatous cell (top and right bottom). Note nerve bundle (left bottom corner). ×9 500

After 30 minutes the lesions of the basal lamina were more conspicuous. The basal lamina was seen to be absent in patches where the cytoplasm was protruding and forming large balloons or bulges giving the area a characteristic appearance (Fig. 9). The balloon like projections often contained organelles such as mitochondria (Fig. 10) cisternae or free ribosomes.

The mesenchymatous cells showed a dramatic change reflecting the enhanced amoeboid activity of these cells. There were numerous branching and non branching protrusions emanating from the cells and affecting one pole of the cell (Figs 8 and 9) only. The cells acquired a bizarre shape and were seen to be linked by slender processes. Such cells were called ropalocytes (Ghadially & Skinnider, 1971).

Treatment with trypsin resulted in swelling also of the endoplasmic reticulum and of the Golgi vesicles.

Nevertheless further development was fairly normal and the overall cellular arrangement regular. The desmosomes appeared to be holding fast though there were some distorted intercellular spaces which contained amorphous electron-opaque matter.

The luminal surface of the acinar cells had many microvilli covered by a spiky glycocalyx. Attachment in the region of the junctional complex tends to be maintained, important for reaggregation. Microfilaments were present forming bundles along the basement membrane in the basal portion of the cells or alongside the outer cell membrane. Similarly microtubules

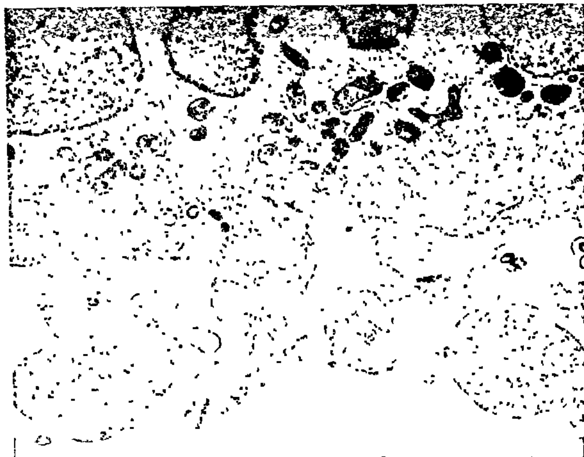


Fig 7 Trypsinized culture (following 30 minutes soak) Basal lamina d srupted or missing and there are many large cytoplasmic protrusions stuffed with ribosomes Some contain cisternae of the endoplasmic reticulum 16 700

were often observed both in the acinar cells and in some nerves

Microfilaments with condensations of Z like material were occasionally seen near the base in some? myoepithelial cells

Innervation

It may be mentioned here that this has been only touched upon by some workers but Garrett s excellent work (1965 1966 and 1972) in this field contributed greatly to our knowledge The innervation of salivary glands was first studied under the electron microscope by Scott & Pease (1959) who have suggested that nerve endings were absent in the epithelium of the submandibular gland of the rat in contrast the present observations show that nerves develop profusely in in vitro cultures of the salivary gland (Fig 11) There are usually large bundles of axons surrounded by a simple Schwann cell lying in the mesodermal layer underneath the basal lamina (Fig 11) The nerves penetrate into the acinus and form connection en passant with the basal and central cells of the acinus (Figs 11 and 12) Synaptic structures could be seen occasionally Dense bodies described by Garrett have been common (Fig 13) but their nature and significance remain obscure

DISCUSSION

The study of embryonic cells by means of separating or dissociating agents such as trypsin



8 As Fig 7 Note the mitochondria in one of the bul- < protrusions 13 600

and EDTA have contributed a great deal to our understanding of the developmental capabilities and specific properties of cells and tissues (Sigot, 1970)

The fine structure of cells after dissociation with trypsin was studied by Lesseps (1963) using embryonic chick cells This study was concerned mainly with surface configuration and the role of cell surface projections in the aggregation of cells as revealed by electron microscopy Hilfer & Hilfer (1966) described the effect of dissociating agents on the fine structure of embryonic chick thyroid cells (16-day chick embryos) Our results show a definite similarity with those observed by Hilfer et al (1964 and 1966) on the effect of trypsin on the thyroid

As in the thyroid, the morphological changes are not uniform for all the cell types comprising the salivary glands Disorganisation of the ground substance and rupture of cells derived from the mesoderm is obvious after the short soak in the enzyme solution Attachments in the region of the junctional complexes tend to be maintained This is of primary importance in reaggregation Certain changes in response to trypsin depend on the actual age and state of differentiation of the explant. There was an apparent increase in the number of free ribosomes and granules of the same density and size within the confines of the cisternae suggesting that the membranes had actually been ruptured It is interesting to note that Hosick & Strohman (1971) observed tran

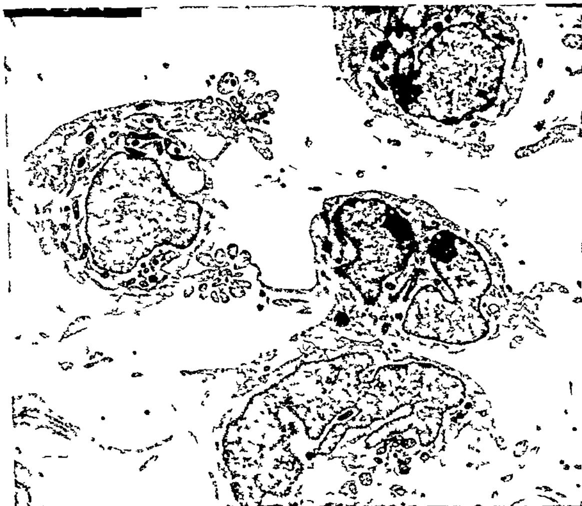


Fig. 9. Trypsinized culture (following 15 minutes soak in trypsin). Ropalocytes showing polarized branching of the outer cell membrane. × 13 600.

sient modifications of polyribosome ratios in chick muscle cells on tissue dissociated by trypsin.

In our material changes of the basal lamina and of the inter cellular ground substance (possibly the outer cell membranes) were conspicuous and merit great interest.

The role and terminology of the basement membrane often confused and confusing has been discussed by Hodges (1969). The basement lamina is an ubiquitous layer situated between various epithelia and underlying tissues. In classical histology it is the PAS positive zone but the basement membrane of the electron microscopist is a 300 Å thick electron-dense layer—the electron opaque layer—to be called basal lamina (Goel & Jurand 1968). Fawcett (1966) has drawn attention to the fact that the basal lamina is not a trilaminar structure of the lipoprotein nature. The precise function of the basal lamina is still largely obscure (Hodges 1969).

Electron microscopically the fully developed basement membrane consists of three layers: (i) an electron lucent zone adjacent to the epithelium, (ii) a basal lamina, (iii) a superficial layer containing collagen like fibres.



Fig. 10. Detail of branching surface of a ropalocyte containing mitochondria. ×47 800.

There is scant data about the role of the basement membrane in developing systems. Goel & Jurand (1968) described some important electron microscopic observations on the basal lamina of chick limb buds after trypsin and EDTA treatment. The authors illustrate but do not discuss cell protrusions of ectodermal origin (Figs. 2 and 3). These are similar to the ballooning protrusions observed by us in vitro indicating a loosening of the basal lamina and enhanced cytoplasmic activity of the epithelial cells as well as of the mesenchymal cells of the developing salivary glands. Hodges et al. (1973) have observed that trypsin is localised in the nucleus; this clearly suggests that intracellular penetration of the enzyme may effect certain intrinsic processes of the cell. It has been generally assumed that cells may lose cytoplasm after trypsinization and a loss of antigenic material from the surface has also been reported (Weiss & Coombs, 1963).

In the present material secretory cells showed minor ultrastructural changes but there was considerable variation in the structure of the secretory granules mainly in their electron density. This varied from one granule to another but also within the same granule.

The *connective tissue* cells surrounding each acinus were markedly changed. Cells with swollen vesicles, broken membranes and extruded

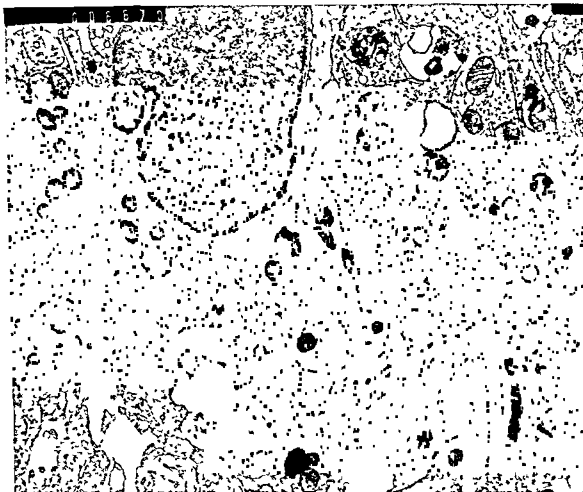


Fig 11 Untreated culture Note many nerve axons passing through basal lamina ×24 200

cytoplasm became evident, leading to the formation of so-called 'ropalocytes' *Ropalocytosis* is the term introduced by Ghadially & Skinnider (1971 and 1972) for cells from a case of "hairy" cell leukaemia Such cells show complex alteration of the cell surface and form numerous branched and non-branched protrusions of the cell membrane In ultrathin sections, such cell processes appear club shaped (*ropalon* = club)

Skinnider & Ghadially (1973) noted such changes in erythrocytes, reticulocytes and normoblasts of leukaemic patients Tentatively it is proposed that ropalocytosis indicates a heightened micropinocytotic and pinocytotic activity The close association of mitochondria with segments of the cell that show this change suggests that they provide the energy needed for the enhanced transport activity

Skinnider & Ghadially (1973) propose that ropalocytosis may be due either to a deficiency of some factor needed by the cells or to the presence of some stimulating agent in the cell environment.

Innervation

There are conflicting views in the literature on the innervation of the salivary glands and in the present study our attention has been focused on the development of innervation both in trypsinized and control cultures Garrett (1972) has reviewed the literature again and has commented "When the literature concerning the



Fig 12 As Fig. 11 Note nerves ending at the base of a cell in the acinus. 20 500

electron microscopy of the innervation of glandular cells is considered it reads like a score card of those seeing axons beneath the parenchymal basement membrane and those not seeing them there".

The presence of nerve endings in direct contact with the acinar cells of the developing gland deserves special notice since fully matured adult acini are almost entirely devoid of nervous elements within the basal lamina Yohro (1971) has described the nerve terminals and cell junctions in young and adult submandibular glands and noted, in full-term embryos, bundles of axons, incompletely wrapped in Schwann cells which may lie in close proximity to the secretory cells of the terminal tubules Axons penetrate the basement membrane around the terminal tubules and straight axons were often observed in the intercellular spaces, which were considered to be growing nerves and most frequently seen within a few days of birth

The changes with age were attributed to acinar growth outstripping nerve growth This work indicates that the age of the material may have an important influence on the innervation patterns in other glands, and should be taken into consideration in innervation studies The presence of *nerves* in the in vitro developing gland was confirmed No specialised cells were found but synaptic structure was present and dense

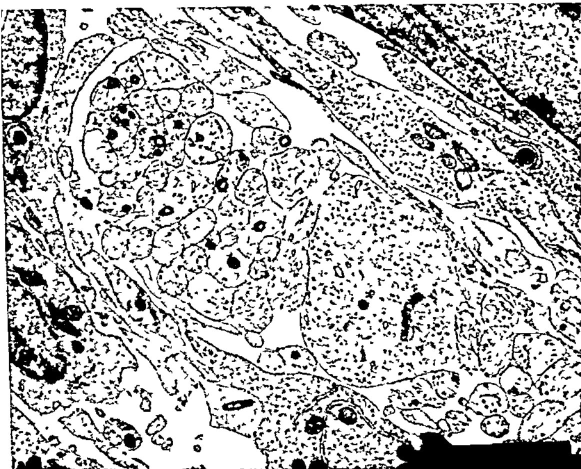


Fig 13 Nerve bundle with dark bodies in many axons × 11 700

structures in some of the axons similar to those described by Garrett (1966) Their significance and character has remained obscure

Walsh & McLelland (1974) described intra epithelial axons in the trachea of the domestic fowl and believe that they play a role in breathing Hung et al (1973) described nerves and associated cells in the bronchiolar epithelium of the mouse lung Whether these are sensory receptor cells, in these organs, i e, neuro epithelial bodies, is not clear The similarity to nerve endings at the neuro-epithelial cells of the ear is however, of some interest

According to Garrett (1972) there is no uniform pattern of innervation of salivary glands, species differences exist, also glandular differences within the same species and cellular differences within the same gland Sometimes there are variations in the extent of innervation from one animal to another in the same species The reasons for these differences are ill understood, but they create the need for an awareness of their existence in physiological experimentation

Cilia with little internal organisation but with two basal centrioles were frequently seen to project irregularly into the lumen or within the acinus, occasionally arising from mesenchymatous cells We have described such atypical cilia in otocyst cultures exposed to toxic agents (Friedmann & Bird, 1971) and others have observed them arising from a great variety of cells

The effect of trypsin may be related to colla-

Drugs

The following drugs were used propranolol hydrochloride ("Inderal", Imperial Chemical Industries, Ltd), atropine sulphate (British Drug Houses, Ltd) and hexamethonium bromide (May & Baker, Ltd)

RESULTS

Stimulation of Nasal Mucous Membrane

Cigarette smoke was passed through the nose for periods of 20 sec and the results of 14 tests in 7 animals are shown in Fig 1 The effect on breathing was either apnoea in the expiratory position or a reduction in respiratory minute volume ($P<0\ 001$) On the cardiovascular system, an increase in pulse interval ($P<0\ 01$) occurred with small and variable changes in blood pressure ($0\ 1>P>0\ 05$) during the period of stimulation As in previous studies, however, a striking after-rise in blood pressure took place following cessation of the stimulus in some of the tests (Angell-James & Daly, 1969*b*, 1972*b*) During the period of stimulation, a fall in total hind limb blood flow ($P<0\ 001$) and in hind limb muscle blood flow ($P<0\ 005$) occurred so that the calculated vascular resistances increased ($P<0\ 01$ and $P<0\ 01$ respectively) These cardiovascular responses are similar to those reported previously with smoke (Angell-James & Daly, 1969*b*, White & McRitchie, 1973, Allison, 1974) and with mechanical stimuli (Tomori & Widdicombe, 1969)

As part of the same series of experiments water or sodium chloride solution (0 9 %) was used as the stimulus to the nose and the results which are similar to those reported here for smoke, have been described in detail elsewhere (Angell-James & Daly, 1972*a*) However, the responses obtained using water or saline and smoke differed in one important respect, namely, in the responses following the application of a local anaesthetic, procaine hydrochloride (2 %), to the nasal mucous membrane It was found that whereas the respiratory and cardiovascular responses to water were abolished, those to smoke in two experiments were not These residual effects consisted of tachycardia, hyperpnoea a rise or fall in blood pressure and an increase in limb vascular resistance (Fig 1) They differed from those to smoke before procaine in that they came on only 15–20 sec after the application of the stimulus and reached a maximum at about 2 min These responses are discussed below

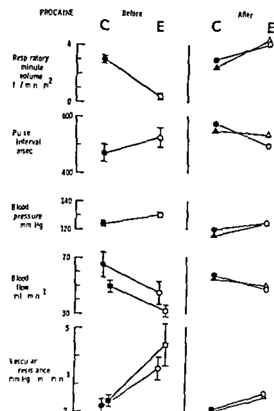


Fig 1 The respiratory and cardiovascular effects of cigarette smoke drawn through the nose The data includes control (*C*) and experimental (*E*) values (means ± S E M) for 14 observations in 7 animals Blood flow and vascular resistance include observations on the intact hind limb (●—○) and skinned hind limb (■—□) The results from two animals are shown after the application of procaine (2 %) to the nasal mucous membrane

Stimulation of Larynx

Since stimulation of the larynx by water and by electrical stimulation of the central end of a superior laryngeal nerve produced qualitatively and quantitatively similar responses, the results obtained by the two methods will be pooled together

Table I. *The effects of stimulation of the larynx by water and of electrical stimulation of the central end of the superior laryngeal nerve on respiration and the cardiovascular system*

The values represent the means ±S E.M , those in parentheses, the range (25 observations in 5 animals)

	Control	Experimental	Difference
Respiratory minute volume (ml/min m²)	3 81±0 17 (2.63±5 74)	0 09±0 06 (0–1 16)	3 72±0 19 (2.63 to −5 74) *P*<0 001
Pulse interval (msec)	691 6±52.5 (280–1 500)	1 092.8±102.3 (600–2 700)	400 8±93 3 (0–2 010) *P*<0 001
Arterial blood pressure (mmHg)	124 4±4 7 *(98–166)*	114 6±5 9 *(60–180)*	−9 1±3 1 (−*42* to −*10*) *P*<0 01
Limb blood flow (ml min⁻¹)	66 6±4 4 (40–125)	41 9+3 6 (21–80)	−24 8±3 3 (−8 to −60) *P*<0 001
Limb vascular resistance (mmHg/ml min)	2 08±1 7 (1 12–3 69)	3 12±0.28 (1 43–5 67)	1 04+0 16 (0 12 3 50) *P*<0 001
Arterial blood			
Po_2 (mmHg)	184 2±15 2		
Pco_2 (mmHg)	43 6±1 2		
pH	7 324±0 01		

In 5 animals, both methods of a stimulation caused inhibition of breathing in the expiratory position or more rarely a reduction in breathing, usually slowing of the heart, a small fall of arterial blood pressure and an increase in hind limb vascular resistance These results are summarized in Table I

Apnoea occurred in 23 of 25 tests, in the remaining two tests there was a reduction of breathing The period of apnoea was variable, lasting 7–45 seconds before the "break-through" occurred

In 22 of 25 tests, the heart slowed as indicated by an increase in pulse interval, in the remaining three tests, the interval did not change Taking all tests into consideration, the pulse interval increased by 69 5 %

Changes in arterial blood pressure were small and rather variable, the average change being a reduction of 8 4 % In about half the tests, cessation of the stimulus resulted in an after-rise in blood pressure of varying magnitude before returning to its original level The highest mean pressure observed was 235 mmHg

Hind limb blood flow invariably decreased by an average of 36 0 %, so that the calculated vascular resistance increased by 52 3 % (Table I) In the absence of appreciable changes in blood pressure, this is interpreted as indicating a predominance of constriction of the "resistance" vessels

Similar cardiac and vascular responses occurred in two animals in which respiration was maintained constant artificially, indicating they were not secondary to apnoea that occurred in spontaneously breathing animals (Fig 2*A*)

The cardio-inhibitory response produced by water to the larynx and by electrical stimulation of the superior laryngeal nerves, was unaffected by propranolol (0 5 mg/kg) but was abolished by atropine (0 2 mg/kg), indicating that it is mediated largely through the vagus nerves These drugs left the respiratory and vascular responses unaffected The latter, however, was abolished by the ganglionic blocking agent, hexamethonium (10 mg/kg) All responses to stimulation of the larynx by water were abolished by division of both superior laryngeal nerves

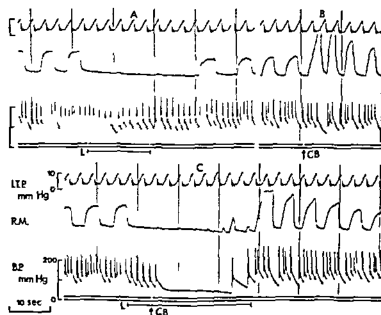


Fig 2 The effects of electrical stimulation of the central end of the right superior laryngeal nerve and of stimulation of the carotid body chemoreceptors by cyanide (7 0 μg/kg) Dog male 14 4 kg Intermittent positive pressure ventilation open pneumothorax *A* superior laryngeal nerve stimulation 3V, 1 msec 15 Hz. *B* carotid body stimulation alone *C*, carotid body stimulation during stimulation of the superior laryngeal nerve A repeat stimulation of the carotid body alone (not shown) had a similar effect to that shown in *B* *ITP* intratracheal pressure *RM*, movements of the ribs (inspiration upwards) *BP*, arterial blood pressure Time calibration 10 sec

Stimulation of Carotid Body Chemoreceptors

In the same 5 animals 16 tests of stimulation of the carotid body by injection of sodium cyanide (3 8–8 8 μg/kg) into a common carotid artery caused, after a latency of 0 75–2 0 sec, an increase in *respiratory minute volume* of 6 03±0 92 l/min m² (range 1 21–12 3), the initial control value being 3 69±0 19 l/min m² (range 2 75–06) This represents an average increase in te volume of 167 5 % ($P<0\ 001$)

pulse interval either increased (10 tests) remained unchanged (6 tests) Bradycardia represents the primary cardiac response to stimulation of the carotid bodies, and the reason it was not observed in every test is that it is opposed by secondary mechanisms resulting from the concomitant increase in respiratory minute volume (Daly & Scott, 1958) Considering all the 16 tests together, there was an increase in pulse interval of 128 1±46 7 msec (range 0–660) or 19 3 %, the control value being 701 9±57 5 msec (range 250–1 200) ($P<0\ 02$)

The mean arterial blood pressure increased by 2 6±1 0 mmHg (range 0–10) from a control value of 117 6±5 1 mmHg (range 95–140) ($P<0\ 05$)

Injection of similar quantities (0 05–0 2 ml) of 0 9 % sodium chloride solution had no effect The responses to cyanide were abolished by division of the carotid sinus nerve, thereby demonstrating their reflex nature, and are similar to those reported previously by Angell James & Daly (1973)

Combined Stimulation of Larynx and Carotid Body

In 16 tests on 5 animals the carotid body was stimulated during the period of excitation of the laryngeal mucosa by water or of a superior laryngeal nerve, and all the results are summarized in Fig 3

Respiration

The hyperpnoea which occurred with control injections of cyanide was either abolished (12 tests) or reduced in size (4 tests) when the injections were repeated during stimulation of the larynx or superior laryngeal nerve Records from one such experiment are illustrated by Fig 2 Whereas the control stimulation of the carotid body alone (*B*) caused an increase in respiration (indicated by movements of the ribs) injection of the same dose of cyanide during stimulation of the superior laryngeal nerve had no effect on respiration (*C*)

Comparison of all tests of stimulation of the carotid body alone with those during excitation of the larynx shows that the respiratory minute

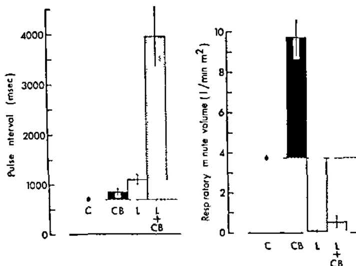


Fig 3 The effects of stimulation of the carotid body chemoreceptors alone (*CB*, filled blocks) and during excitation of receptors in the larynx or of a superior laryngeal nerve (*L+CB*) on pulse interval and respiratory minute volume Open blocks (*L*) stimulation of the larynx alone The filled circles (●), *C*, are the control values The values represent the means ±S E M from 16 series of observations in 5 animals breathing spontaneously

volume increased by 6 03±0 92 l/min m² and 0 56±0 41 l/min m² respectively A paired analysis of the data indicates that this difference is statistically highly significant ($P<0\ 001$)

Pulse interval

In 10 of 16 tests in which stimulation of the carotid body alone caused an increase in pulse interval, the response was greatly enhanced when the test was repeated during stimulation of the larynx In the 6 control tests in which cyanide had no effect on pulse interval, an increase occurred when each test was repeated during excitation of the larynx

The striking differences in the cardiac responses to carotid chemoreceptor stimulation, which were consistent in all experiments, are shown in Fig 2 The control injection of cyanide in *B* led to an increase in pulse interval measured during the expiratory phase of breathing from 1 500 to 2 610 msec When the same dose was repeated during the period of electrical stimulation of the superior laryngeal nerve (*C*) it resulted in cardiac arrest lasting 19 2 sec

The results of 16 tests in 5 animals are shown in Fig 3 The pulse interval resulting from stimulation of the larynx or of the superior laryngeal nerve alone was 1 092 8±102 3 msec (range 580–2 700) and the superimposed stimulus of the carotid chemoreceptors increased this value by 2 808 7±580 6 msec (range 560–6 650) to 3 943 7±614 5 msec (range 1 200–8 000) This represents an increase of pulse interval of 257 %, compared with the value of 19 3 % when the chemoreceptors were stimulated alone This absolute value for the increase in pulse interval was 22 times greater than that of 128 1±46 7 msec ($P<0\ 001$)

When compared with the control value for pulse interval it was found in all 16 series of observations that the cardiac responses to combined stimulation of the chemoreceptor and laryngeal receptors were much greater than the sum of their separate effects This is also evident from the averaged results of the five experiments shown in Fig 3 and from the two experiments in which ventilation was artificially maintained constant (Fig 2)

Arterial blood pressure

In individual series of observations, no consistent relationship was found between the change in blood pressure and the change in pulse interval on stimulation of the carotid chemoreceptors either alone or during stimulation of the larynx When the data is grouped, a fall in pressure of 45 2±7 3 mmHg occurred during combined stimulation, compared with a rise of 2 6±1 0 mmHg during stimulation of the carotid body alone

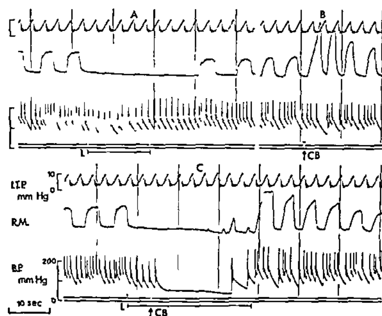


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DISCUSSION

M de Burgh Daly Mr Chairman, Members of the Collegium Thank you very much for allowing me to open this discussion. I would like to make a few general remarks about the general concept of the interaction of cardiovascular reflexes which was the basis of the study which Dr Jennifer Angell James has just presented.

The classical method of studying cardiovascular reflexes has been to isolate the region containing the receptors under study to subject the receptors to a controlled physiological stimulus, and to measure the changes in heart rate blood pressure regional blood flow and so on In engineering terms this is an "open loop" servo-control system and studies of this sort have given valuable information about the control of the circulation by specific groups of receptors. Nevertheless it is somewhat unphysiological in that only rarely does the central nervous system receive a change in the sensory input from one specific group of receptors This is because stimulation of one group produces a change in a cardiovascular or respiratory parameter which in turn leads to a change in another sensory input, and so on, leading to a chain reaction involving other receptor groups, changes in blood levels of hormones and alterations in blood gas tensions and pH Additional information about the way in which the central nervous system deals with changes in two or more sensory inputs simultaneously is therefore required to enhance our understanding of the integrative mechanisms involved in the control of the circulation.

We have already shown (Angell James & Daly, 1969, *J Physiol 201*, 87) how the cardiovascular responses to stimulation of the carotid and aortic body chemoreceptors can be strikingly modified by simultaneous excitation of receptors in the lower respiratory tract. Now, today, we have presented evidence that there is a potent interaction of reflexes from receptors in the upper respiratory tract and carotid bodies affecting respiration and heart rate But what to us is the more fundamental, is that the input from the upper respiratory tract receptors affects the chemoreceptor respiratory and cardiac reflexes in different ways. Whereas the carotid body respiratory reflex is inhibited by afferent impulses from the nose (Angell James & Daly, 1973, *J Physiol 229*, 133) and from the larynx, the carotid body cardio-inhibitory reflex is greatly enhanced by impulses from both groups of receptors

Another point worth mentioning is that the responses elicited by stimulation of two different groups of receptors do not necessarily sum algebraically when the receptor groups are stimulated in combination. We have presented an example of this in connection with stimulation of the larynx and carotid bodies. There is a six fold increase in the response to combined stimulation compared to the sum of their separate effects. This fact together with the observation that the carotid body respiratory reflex response is inhibited by nasal and laryngeal stimulation is what makes the combination of excitation of receptors in the upper respiratory tract and asphyxia so much more potentially dangerous.

As a final point, I would like to mention something about the function of reflexes elicited from the upper respiratory tract. These may be considered as being twofold Firstly, these reflexes serve a *protective* function preventing fluids and irritant gases from entering the lungs by inhibition of respiration and laryngeal spasm. Secondly, they might be considered, in some species at least, to serve a *purposeful* function in that together with reflexes from the arterial chemoreceptors they bring

man can only hold his breath for up to about 2 minutes. References to this aspect of upper respiratory tract reflexes are to be found in our full paper The so-called diving response in aquatic mammals is achieved by slowing of the heart, a reduction in cardiac output and also arteriolar vasoconstriction so intense that the peripheral circulation virtually ceases. Blood only circulates therefore through the heart, lungs, and coronary and cerebral circulations. Reflexes from the face upper respiratory tract and

peripheral arterial chemoreceptors play an important role in bringing about these cardiovascular changes (see Angell James & Daly, 1972, *Soc exp Biol symp 26*, 313) In man too, face immersion in water combined with breath holding, leads to bradycardia and vasoconstriction (see Daly & Angell James (1974) in press) Other clinical aspects of upper respiratory tract reflexes are described in our full paper

J Angell James Since these reflexes have such a profound and vital effect, is it possible that deaths reported suddenly during applications of local anaesthesia and manipulation of the nose and larynx may have been attributable to them rather than to the toxic effects of the drugs? This is yet another reason for stressing the supreme importance of the utmost gentleness in such procedures

B H Colman Were the effects described entirely due to the effect of smoke, or might some of them be due to the effects of special components of cigarette smoke, some of which might be absorbed via the nasal mucous membrane? In respect of the cardiac and vasoconstrictor effects, had adaptation effects been observed in cases of chronic or of recurrent exposure?

G I Schulthess Complications after application of vasoconstrictory nose drops to infants are well known Are these due to reflex or to resorption of the vasoconstrictor?

D F Harrison Since the purpose of most human reflexes is protection, is the nasal reflex described associated with laryngeal spasm? What evidence is there that physiological experiments on a quadruped, such as the dog, relate to the human biped? This particularly relates to the results reported in this paper

F Escher Can these reflexes be trained especially for diving?

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To Mr *Schulthess* It is difficult to be too specific about the mechanism involved in this situation but one [illegible] and those of others that [illegible] sensitive to stimulation of the upper respiratory tract. In addition, drugs are known to be rapidly absorbed through the nasal mucous membrane, as shown by the late Dr Malcomson (1969, *J Laryngol Otol 73*, 73) who [illegible] and larynx does produce reflex spasm of the larynx (Szereda Przestaszewska & Widdicombe 1973 *Respiration Physiol 18*, 107)

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To Mr *Escher* There are certainly ways in which the breath hold time in man may be extended, for instance by breathing 100% oxygen immediately before diving, by a short period of voluntary hyperventilation to lower the level of the arterial blood P_{CO_2} or by a combination of the two But we do not know of any evidence which would indicate that the reflexes we have described can be 'trained' for prolonging the diving times "Biometrics" have been used to control heart rate and blood pressure in man but I am not aware this technique has been used in the control of laryngeal reflexes

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Acta Otolaryngol 79 253–258, 1975

THE INFLUENCE OF OPTOKINETIC TRAINING UPON VESTIBULAR HABITUATION

OK-induced Modification of Vestibular Responses Elicited by Sinusoidal Stimuli

C R Pfaltz and Y Ohtsuka

From the Department of Otorhinolaryngology, University of Basel Basel, Switzerland

Abstract Unidirectional optokinetic training modifies the response pattern of bidirectional sinusoidal vestibular stimuli (pendular rotation) resulting in an initial response increase which is followed by a progressive response decline The latter affects only slow phase velocity, whereas the former shows a frequency increase Optokinetic training obviously has a facilitating effect upon vestibular habituation which cannot be induced by repeated sinusoidal vestibular stimuli alone Peak frequency increase as well as slow phase velocity decrease of vestibular nystagmus induced by optokinetic stimuli, are interpreted as a typical transfer mechanism across two sensory systems

Nystagmus of vestibular origin is known to be influenced by habituation resulting in a progressive response decline In a recent experimental series carried out in normal human test subjects (Pfaltz & Kato, 1974) we have attempted to investigate the influence of unidirectional optokinetic training upon the response pattern of bidirectional vestibular stimuli (angular acceleration) The results of our experiments in normal human test subjects indicate that optokinetic training, i e repeated visual stimuli, facilitates the development of vestibular habituation (response decline)

Greiner et al (1970) studied vestibular habituation in normal subjects with the help of pendular stimulation They came to the conclusion that in healthy persons habituation of the vestibular system cannot be induced by means of repeated sinusoidal stimulations They explained this observation by the following hypothesis The more physiologic the stimulus, the less probable the occurrence of habituation, because physiologic stimuli seem to avoid the maximum of non-vestibular influences, particularly those of central origin Janeke et al (1971) instead of this gave the explanation that the swing movements were too small to provoke habituation

Continuing our study of the interaction between the visual and the vestibular system in respect to habituation we have attempted to answer the following questions

1 What is the influence of optokinetic training upon the response pattern induced by repeated torsion swing stimuli?
2 Why does vestibular habituation not occur after repeated sinusoidal pendular stimulations?

METHOD AND EXPERIMENTAL PROCEDURE

We examined 2 groups of normal subjects

Group A 5 healthy young persons, unidirectional optokinetic training target movement clockwise (CW) producing an optokinetic nystagmus with its rapid phase beating in an anticlockwise (ACW) direction This training was followed by repeated pendular rotation test (PRT) trials

Group B 5 healthy young persons, who were not submitted to an optokinetic training but

This research project was supported by the Schweiz. Nationalfonds zur Förderung der wissenschaftlichen Forschung.

peripheral arterial chemoreceptors play an important role in bringing about these cardiovascular changes (see Angell James & Daly, 1972, *Soc exp Biol symp 26*, 313) In man too, face immersion in water combined with breath-holding, leads to bradycardia and vasoconstriction (see Daly & Angell-James (1974) in press) Other clinical aspects of upper respiratory tract reflexes are described in our full paper

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[illegible]

in origin from the nasal mucous membrane because they are abolished by local anaesthesia The late effects, however, demonstrated after the application of a local anaesthetic to the nasal mucosa, are in all probability due to systemic absorption

We have not repeated tests of nasal or laryngeal stimulation in chronic experiments on dogs in which recovery took place between experiments. In our acute experiments, however, repeated tests at intervals during the course of any one experiment led to reproducible results Prolonged stimulation will lead to asphyxia and hence to stimulation of the carotid bodies with effects we have described in this paper Eventually respiratory 'break-through" must occur which through various secondary respiratory mechanisms will lead to variable results described elsewhere (Angell-James & Daly, 1969, *J Physiol 201*, 87)

To Mr *Schulthess* It is difficult to be too specific about the mechanism involved in this situation, but one might surmise from our results and those of others that a nasal reflex could be involved Infants are more prone than adults to cardiac irregularities initiated reflexly and it might be anticipated that they would be particularly sensitive to stimulation of the upper respiratory tract. In addition, drugs are known to be rapidly absorbed through the nasal mucous membrane, as shown by the late Dr Malcomson (1969, *J Laryngol Otol 73*, 73) who was consultant here in Bristol, so that a general systemic effect could be involved as well

To Mr *Harrison* Yes, stimulation of the nasal mucosa and larynx does produce reflex spasm of the larynx (Szereda Przestaszewska & Widdicombe 1973, *Respiration Physiol 18*, 107)

We appreciate of course that caution is necessary in transferring the results obtained on animals to man. But there is now considerable experimental evidence, cited in our full paper, indicating that the respiratory and cardiovascular reflexes elicited from trigeminal nerve receptors and from receptors in the larynx can be elicited in the cat and rabbit (Kratschmer, 1870, *Sber Akad Wiss, Wien 62*, 147) as well as in the dog, and are similar in man, though probably less potent The responses from arterial chemoreceptors are also qualitatively similar in man (Guz, Noble, Widdicombe, Trenchard & Mushin, 1966, *Respiration Physiol 1*, 38) But so far as we know no attempts have yet been made to demonstrate in man the interactions between reflexes from the upper respiratory tract and carotid bodies such as we have described in the dog

To Mr *Escher* There are certainly ways in which the breath hold time in man may be extended, for instance by breathing 100% oxygen immediately before diving, by a short period of voluntary hyperventilation to lower the level of the arterial blood P_{CO_2} or by a combination of the two But we do not know of any evidence which would indicate that the reflexes we have described can be "trained" for prolonging the diving times 'Biometrics" have been used to control heart rate and blood pressure in man but I am not aware this technique has been used in the control of laryngeal reflexes

To Mr *Padovan* The only recording systems we have used in this study concern the measurements of respiratory and cardiovascular parameters by means of modern conventional electronic systems Concerning the response evoked by stimulation of receptors in the larynx, the upper cuffed tube was placed in the pharynx about 2 cm above the vocal cords All the responses were abolished by division of both superior laryngeal nerves In other experiments in which we electrically stimulated the central end of a divided superior laryngeal nerve, similar responses were produced

increase after the first PRT-trial, which is maintained for one month

Slow phase velocity

Absolute figures do not reveal a conspicuous response decline in either group A or B However, if instead of this we use reference figures which were analysed statistically, we are able to find a significant difference between group A and B (level of significance <0 05 >0 02)

Reference figures are calculated as follows PRT-trial I, serves as initial value and subsequently as a zero reference figure Results of following trials II–IV are compared with the former The differences with a positive sign indicate a response increase, those with a negative sign a response decline Group A (Fig 3) shows for each trial a trend towards the negative side and the standard deviation of the arithmetic mean is considerably smaller than in group B

A comparison between the arithmetic mean of the results of each trial within the same group does not reveal any statistically significant difference (*t* test level of significance >0 5 <0 6), whereas a statistical analysis of the respective values between Group A and B shows a level of significance >0 2 <0 50

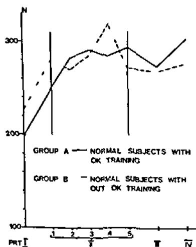


Fig 2 Response increase (Parameter Total number of beats) after repeated PRT Maximum intensity of sinusoidal stimulus 15° s ¹

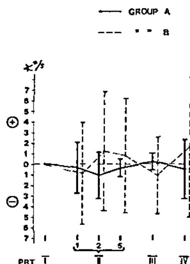


Fig 3 Response decline with respect to eye velocity, occurring only after OK training (15° s ¹ maximum intensity of sinusoidal stimulus)

Hence, our results indicate a true *response decline* with respect to the parameter of *slow phase velocity* which occurs only in subjects previously trained by repeated optokinetic stimuli We have to emphasize that *unidirectional* OK training always induced a vestibular response decrement in *both directions* Because this response decline was not observed in subjects who were only exposed to repeated PRT-trials, we may assume that the visual system is mainly responsible for the development of vestibular habituation

Peak frequency (Fig 4)

Fig 4 shows a definite response increment with respect to this parameter, occurring only after optokinetic training Statistical evaluation of the results obtained in group A and B reveal a level of significance <0 001

Duration of turning sensation (Fig 5)

No difference between group A and B could be demonstrated by means of this subjective parameter

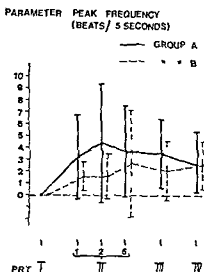


Fig 4 Response increment with respect to peak frequency This modified vestibular response is more conspicuous after OK training than after sinusoidal stimulation alone (15° s⁻¹ max intensity)

DISCUSSION

The most important findings resulting from this habituation study are the following

On the one hand there is a *response increment* of OKN with respect to both frequency and velocity of the slow component as well as an increase of the vestibular responses with respect one single parameter, namely peak frequency

On the other hand there is a conspicuous *response decline* of vestibular nystagmus with respect to the parameter of slow phase velocity Modifications of both optokinetic and vestibular response patterns are entirely due to the influence of repeated visual stimuli

Response increment of OKN has been interpreted in previous publications (Miyoshi et al 1973) as the result of a sensitivity increase of the visual system, subserving its ability to pick up the periodicity of simple motion patterns in order to use this sensory information for an improvement of the motor output Peak frequency increase of vestibular nystagmus, elicited by PRT, is also entirely due to the influence of repeated OK-stimuli This phenomenon may, therefore, be correlated with visual vestibular coordination Cohen & Henn (1972) have shown that both saccades and quick phases of nystagmus in a horizontal plane, as well as slow eye movements and positions of fixation in a horizontal plane are generated in the paramedian pontine reticular formation Hence, it follows that OK and vestibular nystagmus are generated in the same anatomical substratum of the reticular formation The increase of peak frequency of vestibular nystagmus in PRT, due to repetitive OK stimuli, may therefore be explained by a *transfer mechanism* between the two sensory systems which takes place at a common nystagmogenic centre within the pontine region As long as this frequency increase does not interfere with the coordination of visual and vestibular information vestibular nystagmus is not inhibited In the circumstance of a *regular sinusoidal* sensory input the visual system is able to perceive the periodicity of a motion pattern, predicting it to a certain extent and it is also able to correlate visual and vestibular messages to the oculomotor output without difficulty (Kornhuber, 1969) Using, instead of this, *constant angular acceleration* stimuli, the dynamic response of the vestibulo ocular system improves as that of the visual tracking system fails (Melville Jones, 1965), because the intensity of the vestibular stimuli is much higher and this type of stimulation also lacks a regular and predictable periodicity of motion For those reasons visual and vestibular information becomes contradictory and as a consequence vestibular responses decline Thus, vestibular habitua

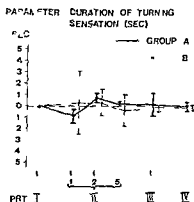


Fig 5 Duration of turning sensation as a parameter does not reveal any response modification following both OK training and repeated vestibular stimulation

tion sets in, opposing inappropriate responses by compensatory reactions, which are associated with visual-vestibular conflicts and exposure to unusual motion environments (Young & Henn, 1974)

Contrary to the reports of Van de Calseyde et al (1969), Greiner et al (1970), Janeke et al (1971) as well as Gonshor & Melville Jones (1969), who were not able to demonstrate any response decline after repeated exposure to sinusoidal rotational stimuli, we found a definite decrease of slow phase velocity of vestibular nystagmus which was however exclusively due to an unspecific optokinetic training As in our previous study on vestibular habituation (Pfaltz & Kato, 1974) this response decline was bidirectional although OK-stimuli were unidirectional

According to Gonshor & Melville Jones (1969) natural patterns of rotational movement do not induce progressive changes of the kind to be expected from previous habituation studies, which are perhaps due to movements sufficiently unnatural to generate misleading sensory information We were able to confirm this statement, as prolonged exposure to repeated sinusoidal rotational stimuli alone did not result in a response decline On the other hand we are not able to explain why OK training alone should initiate a vestibular response decrement We could already observe this phenomenon in a previous habituation study (Pfaltz & Kato, 1974), using angular acceleration stimuli of low intensity (0 05° s^{-2}, applied for 300 sec) In both experimental circumstances the intensity of the *vestibular stimulus* was *low and unable to induce* vestibular habituation without prior optokinetic training For the time being we restrict ourselves to the conclusion that the results of the present study confirm the statement of Young & Henn (1973), whereupon exposure to a purely non-vestibular input leads to vestibular habituation and transfer of habituation across sensory modality is a proof of its central origin

RÉSUMÉ

La stimulation optocinétique unidirectionnelle repétée produit une modification des reponses vestibulaires declenchées par stimulation angulaire sinusoidale repétee Les reponses modifiées sont caracterisées par une augmentation initiale de la fréquence nystagmique suivie par une diminution progressive de la vitesse de la phase lente Ces modifications sont exclusivement provoquees par une stimulation non specifique à l'egard du systeme vestibulaire même Il en ressort que l élèvation de la fréquence aussi bien que la décroissance de la phase lente du nystagmus vestibulaire, produites par une stimulation non spécifique représentent la manifestation d un mécanisme de transfer entre 2 systèmes sensoriels et différents phénome typique de l habituation

ZUSAMMENFASSUNG

Wiederholte optokinetische Reizung in einer Richtung (unidirektionelle Stimulation) fuhrt zu einer Modifikation der durch wiederholte bidirektionelle Reizung (sinusoidale Drehreizung) ausgelosten vestibularen Reizantwort Diese ist gekennzeichnet durch einen initialen Anstieg der Nystagmusfrequenz dann folgt eine progressive Abnahme der Geschwindigkeit der langsamen Phase Diese Änderungen der vestibularen Reizantwort werden ausschliesslich durch *unspezifische optokinetische* Reize verursacht Aus diesen Untersuchungsergebnissen wird gefolgert dass sowohl Anstieg der Kulminations frequenz als auch Abfall der Geschwindigkeit der langsamen Phase des vestibulären Nystagmus, ausgelost durch unspezifische Reize Ausdruck eines Transfermechanismus zwischen 2 Sinnessystemen sind (Habituationseffekt)

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DISCUSSION

M Arslan Which is the anatomical level in which integration takes place between the two nystagmogenic inputs, the vestibular one and the optical?

G F Greiner I think that as we did our investigations in darkness, eliminating visual stimuli, we had only a specific stimulation and bilateral bi sinusoidal movements As Arslan said, the unspecific stimulation introduces other factors and on a different level and therefore give habituation These investigations could have also a practical application for vestibular patients for treatment of vestibular outfalls by OK stimulation

C R Pfaltz (Reply) to Mr *Arslan* The integration centre is most probably located at the level of the para median part of the pontine reticular formation, as shown by Cohen & Henn (1972)

To Mr *Greiner* We could confirm your previous observations, and habituation after repeated sinusoidal stimulation can only be achieved by optokinetic training The visual system therefore takes the leading part with respect to the achievement of vestibular habituation

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NORMAL AND PATHOLOGICAL ADAPTATION OF COMPOUND VIII NERVE RESPONSES IN THE GUINEA PIG

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Abstract Adaptation of VIII nerve compound action potentials in response to trains of broad frequency spectrum clicks and high frequency filtered clicks is studied at various intensities in normal guinea pig under normal conditions, while masking with white noise and under pathological conditions after ototoxic antibiotic treatment. The results are discussed with respect to the clinical electro-cochleographic adaptation studies in man and the so-called two populations of receptors and nerve fibres in the cochlea

Fast adaptation of compound VIII nerve responses to trains of clicks has been simultaneously studied in the guinea pig and in man under normal and pathological conditions

Although studies in human electro-cochleography did not show different, clear-cut, adaptation behaviours in the various pathological conditions (Charlet de Sauvage & Aran, 1974), the main observation was that in pathological human ears, whenever adaptation is abnormal, it is less pronounced than in normal ears (Fig 1) These findings, suggesting that fast adaptation is a very basic and sensitive phenomenon, were studied and interpreted further in the following animal experiments

MATERIAL AND METHODS

Recordings were performed on awake guinea pigs equipped with an electrode permanently implanted on the round window The reference and ground electrodes were taken on the screws placed on the skull to hold the electric plug where the three electrodes are soldered The methods for electrode implantation and for the delivery of the stimuli at the ear-drum in the awake guinea pig (via a plastic tube, fitted into the internal auditory canal, passing through the plug on the skull and joining a TDH 39 Telephonics receiver) have been already described and used in various experiments (Portmann et al, 1966, 1973, Stephens et al, 1974*a* Aran & Darrouzet, 1974) Such preparations are quite reliable since VIII nerve responses, normal in every respect, have been recorded in several such guinea pigs for by now more than two years

Stimuli

The stimuli always consisted of repetitive trains of 5 clicks or filtered clicks of alternate polarity (to cancel out the cochlear microphonic in the averaged response) Clicks were produced by 0 1 ms rectangular impulses sent into the Telephonics receiver Filtered clicks were made of 0 062 ms rectangular impulses filtered into a band pass (7 200 to 8 800 Hz) passive filter (30 dB/octave) centred then on 8 000 Hz The silent interval between trains was always of 100 ms Interclick intervals were 8 5, 16 5 and 25 5 ms for studies in the normal guinea pig while, after this study in the normal, only an 8 5 ms interclick interval was used for studies in pathological guinea pigs and for masking experiments (as well as for the human electro-cochleographic

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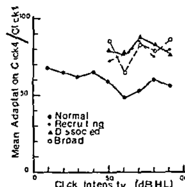


Fig 1 Adaptation of electro-cochleographic responses (click 4/click 1) in human ears with normal and various pathological responses patterns as a function of intensity (Charlet de Sauvage & Aran, 1974)

measurements, Charlet de Sauvage & Aran, 1974)

Trains of 5 stimuli were used because former adaptation studies in the guinea pig (Eggermont & Spoor, 1973, Stephens et al, 1974*a*) as well as in man (Yoshie & Ohashi, 1971, Eggermont et al, 1974, Stephens et al, 1974*b*) have shown that adaptation was completed after the 5th click of the train Stimuli were presented from threshold to 80 or 90 dB by steps of 10 or 5 dB The zero reference level is the intensity corresponding to the mean Visual Detection Level of averaged response in Normal guinea pigs (dB HL)

Signal acquisition

Every other responses to the successive trains of alternated clicks were averaged in two different memories, using a 40 ms window (1 024 memory points) Using an odd number of stimuli in the trains (5), the clicks, alternated from one to the other, were in opposite phase in each averaged response to the trains Then adding and substracting the two responses gave respectively the Action Potential (AP) responses alone or the Cochlear Microphonics (CM) alone (although sometimes superimposed to the unsymmetrical elements of the AP responses to the condensation and rarefaction clicks) Such CM recordings were used to control the intensity of each click in the train CM was indeed constant and did not show adaptation

Measurements

Amplitude of N1 from the foot of AP to the negative trough was measured on the 5 responses and expressed either, for responses 2 to 5, in % of the amplitude of the response to the first click of the train (adaptation curves), or in % of the maximum amplitude of the response to the first click (at 80 or 90 dB) (input–output amplitude functions) (Figs 2 and 3)

Myogenic responses were never observed in the various recordings Then amplitude measurements could be more accurate, particularly for responses to the second click, than in man where a positive signal of around 12 ms latency was very often superimposed on the AP responses Apparently this response was not related to middle ear muscles contraction as evidenced by the constant amplitude of CM (Charlet de Sauvage & Aran, 1974)

As in man, owing may be to the limited accuracy provided by the sampling speed used (40 μs/memory point), no significant changes in the latency of the AP responses during adaptation were observed and, although such measurements were systematically performed, the results are not presented here

RESULTS

Normal responses

Mean adaptation in 10 normal guinea pigs is presented in Fig 2 for various interclick intervals (25 5, 16 5, 8 5 ms) for the clicks and 8 000 Hz filtered clicks Mean input–output amplitude functions, using an 8 5 ms interclick interval are represented in Fig 3

These figures illustrate that fast adaptation is a function of the interclick interval and is completed for the 5th click, as it has been shown already by Eggermont & Spoor (1973)

The main point here is that, for adaptation as a function of intensity, there is with the 8 5 ms interclick interval, a marked dip around 70 dB, already evident on the responses to the broad frequency spectrum click and very pronounced for the high frequency filtered click Moreover, this dip occurs at the intensity level

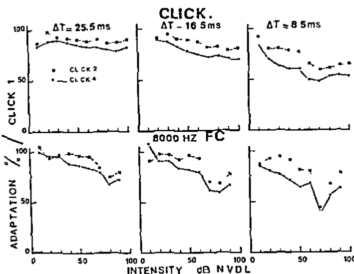


Fig 2 Mean adaptation curves of VIII nerve responses (click 2 and click 4/click 1) in 10 normal guinea pigs for the click (above) and the 8 000 Hz filtered click (under) using various interclick intervals (ΔT)

which corresponds to the plateau—or shoulder—in the growth of amplitude on the input–output functions

These results are absolutely identical with that found in normal human ears (Charlet de Sauvage & Aran, 1974) using same stimuli and interclick intervals (Fig 1, normal)

Pathological responses

Adaptation was similarly measured in 3 guinea pigs treated with an ototoxic antibiotic (Kanamycin), in the course of experiments conducted to complete the preliminary long term studies of such ototoxicity reported earlier (Aran & Darrouzet, 1974)

In these experiments, guinea pigs were given 400 mg/kg body weight i m injections of Kanamycin for 6 consecutive days The responses were recorded during several months after the treatment These experiments are still in progress but adaptation was observed during evolution of the VIII nerve responses to the click which became eventually of the *Dissociated* and *Recruiting* types (Aran, 1973)

Input–output amplitude and latency functions for the click (click 1), and adaptation curves (click 4/click 1) are represented for the same guinea pig, 3 weeks apart while the responses to the unfiltered click, from *Dissociated* (Fig 4, 4 days post-Rx), became *Recruiting* (Fig 5, 24 days post-Rx) The responses to the 8 000 Hz filtered click were already, at both stages, of the *Recruiting* type (Figs 4 and 5)

It must be recalled that human electro-cochleographic studies (Aran & Negrevergne, 1973) and subsequent animal experiments (Portmann et

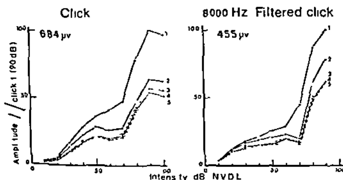


Fig 3 Mean input–output amplitude functions for the 5 clicks of the trains (click and 8 000 Hz filtered click) in 10 normal guinea pigs The respective mean amplitudes are expressed in % of the mean maximum amplitude of the response to click 1, indicated in the left hand corner of each graph

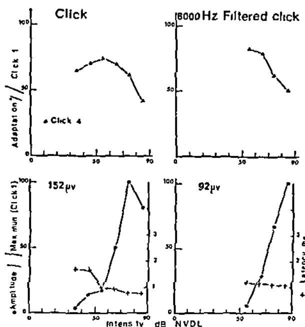


Fig 4 Adaptation curves (click 4/click 1) and input-output amplitude and latency functions (click 1) for the click (left) and the 8 000 Hz filtered click (right) in a Kanamycin treated guinea pig (6 × 400 mg/kg) presenting 4 days post Rx, with *Dissociated* responses to the click and *Recruiting* responses to the 8 000 Hz filtered click

al, 1973, Aran & Darrouzet, 1974) have shown that the dissociated responses to the unfiltered click together with the recruiting responses to the high frequency filtered click correspond on the one hand to high frequency sub-total hearing loss with recruitment and, on the other hand, to outer hair cell loss at the base of the cochlea Moreover *Recruiting* responses to both the click and the high frequency filtered click were observed in cases of hearing loss spread over the entire frequency range (flat audiogram with recruitment) where supposedly there is an extensive outer hair cell loss along the entire basilar membrane Although such a situation has not yet been clearly demonstrated in animal experiments, results of microscopic examination of the cochlea (*Surface preparation*, Engstrom et al, 1964) of the same guinea pig shown as an example above are quite consistent with this hypothesis The cochlear cytogram was realized 50 days after the last adaptation measurements (i e 74 days post-Rx) but while the responses to the click recorded just before sacrifice were still very clearly recruiting (Fig 6) Similar results concerning adaptation, input–output functions and cochlear cytograms were obtained for the two other guinea pigs

Adaptation during masking

A condition somewhat similar to the pathological condition described above can be obtained in normal guinea pig while masking the trains of clicks by a continuous white noise of moderate intensity. With the appropriate white noise level, the responses to the low intensity clicks can be masked while the responses to the high intensity clicks are still clear The high sensitivity mechanism of stimulation of the nerve fibres, related, according to the well known hypothesis, to the outer hair cells of the cochlea, would be ineffective to the click during the masking noise, while the low sensitivity—high intensity mechanism, related to the inner hair cells, would be still activated by the click above the noise level Then in such a condition, adaptation of the unmasked responses should be simi-

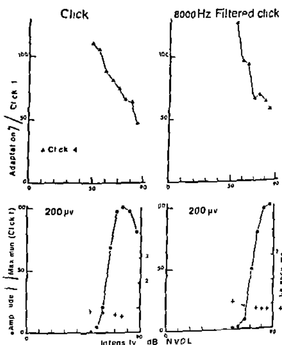
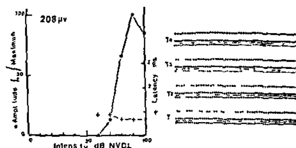


Fig 5 Same as Fig 4 for the same guinea pig presenting 24 days post Rx with *Recruiting* responses to both the click and the 8 000 Hz filtered click



lar to that obtained above in the pathological ears where indeed only the inner hair cells were preserved

Such experiments were conducted in 4 normal guinea pigs The clicks and the noise were presented through two different amplification channels and two different Telephonics receivers connected to the same plastic tube reaching the ear The white noise level was adjusted so that the responses to the clicks were masked from 50 dB and under Adaptation measurements

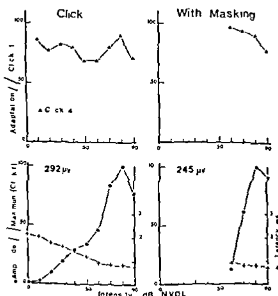


Fig 7 Adaptation curves (click 4 click 1) and input-output amplitude and latency functions (click 1) while the trains of clicks are presented in silence (*left*) and during continuous masking with a white noise whose level *was adjusted so that the 50 dB response was masked* (*right*) in a normal guinea pig During masking the input-output functions are *Recruiting* like and adaptation is less pronounced.

phasic and adaptation is more pronounced This late component of the response, supposedly coming from the nerve fibres activated by the outer hair cells (since it is wiped out by the noise) must be responsible for the pronounced adaptation (since adaptation almost disappears during masking too) It is interesting to note that during adaptation, the pattern of the compound response is not modified (responses 2 to 5 versus response 1) as it is during continuous masking (responses 1 to 5) The fact that both phenomena, adaptation and continuous masking, cause a decrease in amplitude of the compound VIII nerve responses but do not modify the patterns of the responses in the same manner, indicates, as already stated by Eggermont & Spoor (1973), that the mechanisms involved are different

CONCLUSION

These measurements demonstrate that fast adaptation of the compound VIII nerve response is

(1) maximally pronounced at the click intensity level which corresponds to the shoulder in the input–output amplitude function in normal guinea pig,

(2) less pronounced when the outer hair cells extensively impaired,

[illegible]) less pronounced during simultaneous mask[illegible]g while the outer hair cells are supposedly unresponsive to the low intensity clicks

These results are in agreement with similar measurements in normal and pathological human ears (Charlet de Sauvage & Aran, 1974)

They indicate that fast adaptation of the VIII nerve responses is mainly related to the mechanism of excitation of the nerve fibres by the outer hair cells of the cochlea

RÉSUMÉ

L'adaptation du potentiel d action composite du nerf auditif lors de stimulations par trains de clics large bande et de clics filtrés haute fréquence est étudiée a differentes intensites chez le cobaye normal dans les conditions normales, pendant masquage par un bruit blanc, et dans des conditions pathologiques apres traitement par antibiotique ototoxique Les résultats sont discutés en fonction de l étude électrocochleographique clinique de l'adaptation chez l homme et par rapport à la théorie des deux populations de récepteurs et de fibres nerveuses dans la cochlee

ZUSAMMENFASSUNG

Die Anpassung des Potentials zusammengesetzter Aktion des Hornervs bei Stimulationen durch Serien von Breitbandsignalen und Hochfrequenzfiltersignalen wird bei verschiedener Intensitaten am normalen Versuchstier unter normalen Bedingungen wahrend einer Maskierung durch weissen sowie unter pathologischen Bedingungen nach einer Behandlung mit ototoxischen Antibiotika untersucht Die Ergebnisse werden im Hinblick auf das klinische Studium des Elektrocochleogramms der Anpassung beim Menschen und im Vergleich mit der Theorie der beiden Populationen von Rezeptoren und Nervenfasern in der Cochlea diskutiert

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DISCUSSION

H Spoendlin Your work suggests that adaptation depends from the outer hair cells This implies a functional interference between outer and inner hair cell system Do you have any definite ideas how such an interference could take place?

G Djupesland You have studied an input/output function of the cochlea How can you know that the input to the sensory cell does not change during your experiments? Did you eliminate the function of the middle ear muscles?

J-M Aran (Reply to) Mr *Spoendlin* Indeed I am looking to some kind of interaction between outer and inner structures, but about the locus where this happens it is rather you who should tell us!

Mr *Djupesland* About the input to the ear we have many evidences that it does not change, from many measurements in normal guinea pigs where the values are quite constant As far as adaptation is concerned, since we use repetitive trains of clicks with 100 ms intertrain intervals, contraction of middle ear muscles can indeed modify the input to the inner ear, but in an overall way, not during fast adaptation

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RETROGRADE DEGENERATION OF THE COCHLEAR NERVE

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Abstract Retrograde degeneration of the cochlear neurons has been studied in different types and degrees of peripheral cochlear damage such as acoustic trauma, intoxication, heredodegenerative deafness and others It starts only when the peripheral dendrites to the inner hair cells are irreversibly damaged About 10 % of the neurons are not affected by retrograde degeneration They correspond to the type II and III neurons which also survive after transection of the cochlear nerve and are mainly associated with the outer hair cells Cochlear damage due to vascular impairment usually leads to a complete loss of cochlear neurons In hereditary abiotrophic deafness, neuronal degeneration is slower and its extent varies considerably according to the various genetic syndromes

It is well known that retrograde degeneration occurs after destruction of the organ of Corti T fact that an entire neuron degenerates only the terminal portions of its dendrites damaged is a peculiar phenomenon rather ique in the cochlear nerve Especially in respect to electrical stimulation of totally deaf ears, it is important to know what induces this retrograde degeneration and how long and to what extent it proceeds under different pathological conditions For this purpose we produced experimental damage of the end organ by acoustic trauma, intoxication with neomycine or direct surgical destruction and examined the cochlea after different surviving times from a few days up to one year using the bloc-surface technique (Spoendlin, 1974) In addition, the behaviour of the cochlear neurons in hereditary deafness in the deaf white cat and in some temporal bones of human cases with hereditary and acquired total deafness was studied

There have been discussions about the type of alterations in the organ of Corti which induce retrograde degeneration The crucial factor has been proposed to be the loss of inner hair cells (Bredberg, 1968), the collapse of the supporting elements of the organ of Corti (Schuknecht, 1953) or a direct damage to the cochlear nerve dendrites at the inner hair cells (Spoendlin, 1971)

The total loss of outer hair cells over a greater length of the cochlear duct leads to the disappearence of the outer spiral fibres but it has no appreciable effect on the cochlear neurons in the osseous spiral lamina and in the spiral ganglion even after surviving times of one year, as long as the majority of inner hair cells are present (Figs 1 and 2*A*)

The loss of the inner hair cells on the other hand is usually followed by a massive retrograde degeneration of the cochlear neurons, regardless of the presence or absence of the outer hair cells The collapse of the supporting structures does not seem to be crucial for the initiation of degeneration, since complete retrograde degeneration is frequently seen in areas with intact supporting structures (Fig 2*B*) Although there is in general a good correlation between the number of destroyed inner hair cells and the degree of retrograde degeneration, we observed degeneration in areas with the inner hair cells present or no degeneration in areas with extensive loss of inner hair cells This indicates that retrograde degeneration does not depend directly on the presence or absence of inner hair cells, but from some other factors It is most likely the consequence of a direct damage to the peripheral unmyelinated segments of the cochlear neurons associated with the inner hair

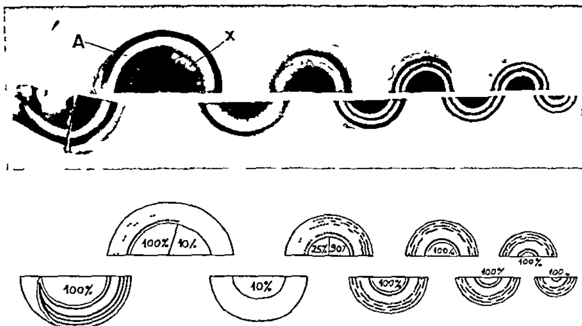


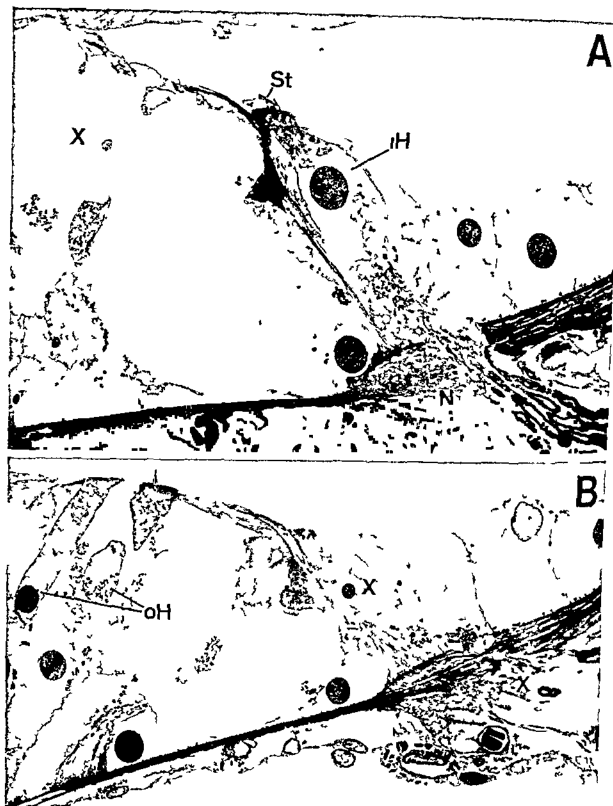
Fig 1 Bloc surface preparation (*above*) with corresponding schematic evaluation (*below*) of a guinea pig cochlea one year following acoustic overstimulation with 140 dB white noise for 20 min In the upper half of the lower basal turn and in the upper basal turn where the organ of Corti is completely gone (blank area) only 10% of the cochlear nerve fibres in the osseous spiral lamina remain (<) In the lower half of the lower basal turn (*A*) where all outer hair cells are missing ( ) but the inner hair cells are present (——) there remains a full population of nerve fibres in the osseous spiral lamina (100%, see Fig 2)

cells as inner radial fibres, which constitute about 95% of the entire cochlear neuron population (Spoendlin, 1969) It has been shown that these inner radial fibres are particularly sensitive to many kinds of injury such as acoustic trauma (Spoendlin, 1971) and anoxia (Spoendlin, 1968), whereas the other nerve fibres are much more resistant

Irreversible damage initiating retrograde degeneration begins with excessive swelling of the fibres and ruptures of the axon membranes This is frequently but not necessarily associated with a loss of inner hair cells or with a collapse of the supporting structures The integrity of its peripheral terminal portion seems to be essential for the neuron to survive

Retrograde degeneration starts almost immediately after rupture of the inner radial fibres and proceeds within a few days through the osseous spiral lamina to the spiral ganglion, where it is considerably delayed After 3 weeks the great majority of nerve fibres in the osseous spiral lamina have entirely disappeared but there is only a slight reduction of ganglion cells in the spiral ganglion It is not before 3 months that degeneration of the ganglion cells has occurred on a larger scale and the number of ganglion cells is drastically reduced in a relatively short time After 5 months only about 10% of the normal ganglion cell population remain and this situation seems to be maintained (Fig 3) After one year there are still about 10% of healthy looking ganglion cells present When only the peripheral receptor is damaged, as in acoustic trauma, mechanical destruction, or intoxication with ototoxic antibiotics, retrograde degeneration never affects all neurons 5–10% of the neurons are always spared and resist retrograde degeneration However, as soon as the cochlear blood supply is impaired or an infection affects the neurons directly, only very few or no neurons survive, resulting in a total loss of neurons

The next question concerns the type of neu-



Fg 2 (A) El ctron m croscop c view of the o gan of Corti from the lo er half of the lo ver basal turn (*A*) of the cochlea in F g 1 Although the outer ha r cells are entirely m s ing ( ) the nerve fibres do not de generate as long as the nner ha r cell (*iH*) are present The outward b nd ng of the sensory ha rs (*St*) of the inner hair cell is a typ cal irr vers ble effect of the acoustical traumat sat on (B) The organ of Cort in the upper basal turn 3 months after acoust c traumat sat on Here most outer ha r cells (*oH*) rema n but the inne ha r cells are m ss ng ( ) and the great majo ity of nerve f bres have degenerated ( )

ron which resists retrograde degeneration In normal guinea pigs (Kellerhals et al, 1967, Spoendlin, 1971) and cats (Spoendlin, 1971, Ross, 1971) two different types of neuron are found in the spiral ganglion The great majority (type I) of ganglion cells are large and myelinated whereas about 10% in the guinea pig and 5% in the cat are smaller and unmyelinated (type II) (Fig 4)

In the guinea pig one year following acoustical destruction of the organ of Corti, the majority of the surviving neurons are small and unmyelinated, corresponding to the type II in the normal ganglion, whereas most of the type I cells have disappeared A similar situation is found in the cat, where also practically all myelinated type I ganglion cells degenerate and disappear in the course of retrograde degeneration The remaining 10% consist partly of type II cells with all their characteristics and partly of another type of ganglion cell which resembles closely the type I but is not myelinated (type III) (Spoendlin, 1973) Surviving type I cells are exceptional

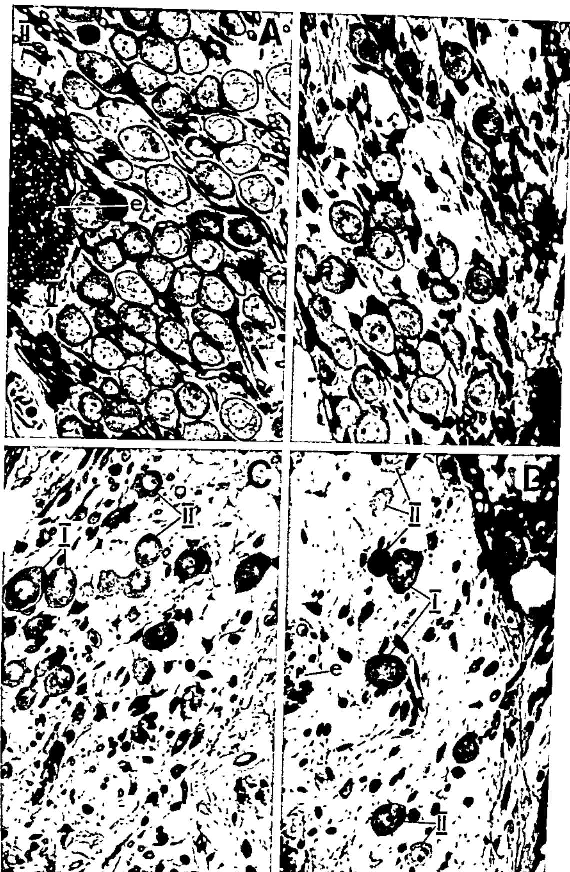
In the cat an interesting comparison between the findings of retrograde degeneration after destruction of the organ of Corti and the degeneration pattern following transection of the cochlear nerve in the inner acoustic meatus may give some hints about the significance of the surviving neurons In fact, the same type of neurons (II and III) resist degeneration after transection of the cochlear nerve (Spoendlin, 1971, 1973, 1974) as in retrograde degeneration Since the organ of Corti does not degenerate after section of the cochlear nerve it is seen that the entire afferent nerve supply of the outer hair cells remains intact and almost all inner radial fibres, the afferent nerve supply of the inner hair cells disappears This means that essentially the afferent neurons for the outer hair cell system resist degeneration and that the type II and III cells belong mainly to the neurons supplying the outer hair cell system, whereas the type I cells belong to the neurons leading to the inner hair cells Since we find the same type of neuron remaining after retrograde degeneration, it is therefore most likely that the surviving neurons after retrograde degeneration following destruction of the organ of Corti also represent mainly the innervation of the outer hair cells, whereas the nerve supply of the inner hair cells degenerates (Fig 4)

It is an open question how much these experimental data in laboratory animals are applicable to the situation in the human There is not much reason to believe that the human cochlea would behave basically differently in damage due to acoustic trauma or intoxication Although antibiotic intoxication is still one of the important causes of accquired total deafness, genetic causes, infections and vascular disturbance play a by far more important role in the aetiology of total deafness and they are hardly reproducible in animal experiments

The histopathology of human temporal bones in persons who became totally deaf following meningitis, bacterial labyrinthitis or head trauma reveals either a total bony obliteration of the cochlea without remaining neurons or, when the cochlear spaces are preserved, a total disappearance of the organ of Corti and a practically total loss of neurons (Fig *5A*, *B*)

Viral labyrinthitis due to mumps or measles, on the other hand affects mainly the endolymphatic epithelial structures and spares the neurons Considerable retrograde degeneration of the cochlear neurons seems to be rare following prenatal and of moderate degree following post natal infections (Lindsay, 1973)

A more complex situation is found in abiotrophic hereditary deafness, which represents a very important cause of total bilateral deafness in man Although histopathological information is rare, it seems that the degeneration process usually begins as a cochleo-saccular degeneration within the endorgan or the stria vascularis with varying degrees of secondary retrograde degeneration of the cochlear neurons Less frequently primary, almost total degeneration of the neurons in the presence of an intact endorgan occurs, as shown in the temporal bones of two profoundly deaf persons suffering from Friedreich's disease (Spoendlin, 1974) In a numb of cases of hereditary deafness with primar



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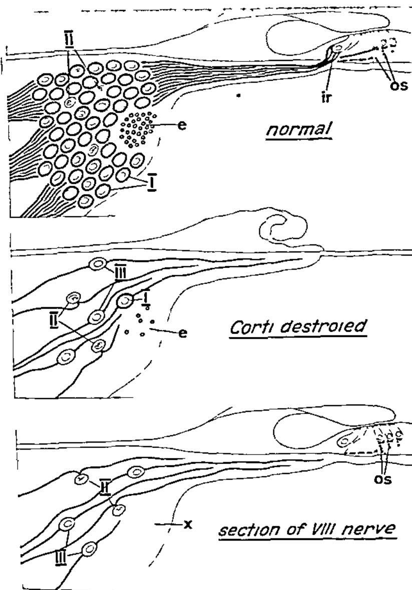
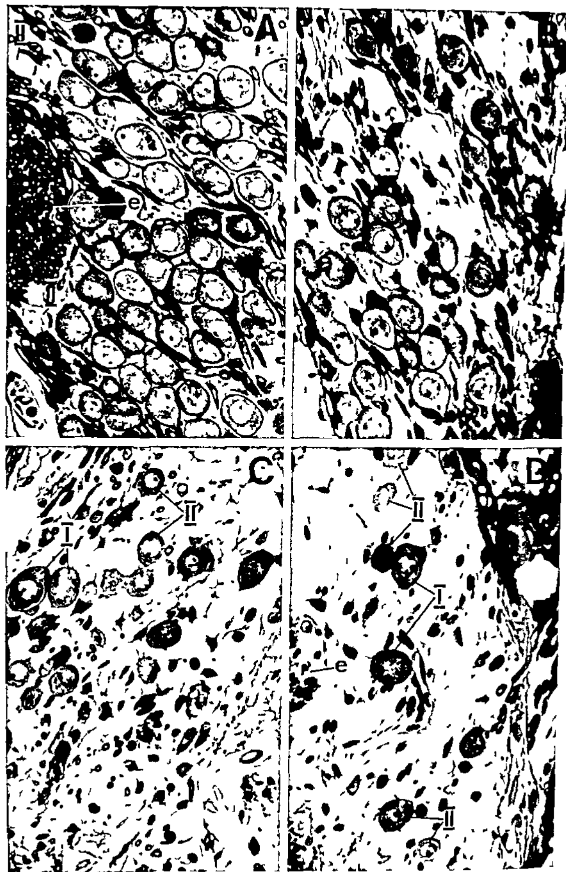


Fig 4 Schematic representation of the cochlear neurons in the cat In the normal cat most ganglion cells are large and myelinated of type I (I) and only few are small and unmyelinated of type II (II) The efferent intraganglionic spiral bundle (*e*) consists of many myelinated and un myelinated fibres, the inner radial fibres (*ir*) provide the afferent nerve supply of the inner hair cells whereas the outer spiral fibres (*os*) are the afferent fibres to the outer hair cells After destruction of the organ of Corti most type I cells degenerate and only type II and III cells remain After section of the VIII nerve the same type of ganglion cells survive together with the outer spiral fibres (*os*) and the afferent nerve endings at the outer hair cells

g 3 Spiral ganglion of the upper basal turn of a guinea pig cochlea (A) Normal situation with a full set of densely packed myelinated type I ganglion cells and a few smaller unmyelinated type II ganglion cells (II) The intraganglionic spiral bundle consists of densely packed efferent fibres (*e*) (B) Three weeks after complete acoustical destruction of the organ of Corti In spite of degeneration of most fibres in the osseous spiral lamina only about 50 % of the ganglion cells have disappeared (C) Three months after acoustical destruction of the organ of Corti Most myelinated ganglion cells of type I are gone (D) One year after acoustical destruction of the organ of Corti 5-10 % of the ganglion cells, mainly type II cells (II) and only few type I cells (I) remain without any signs of degeneration Also the number of efferent fibres in the interganglion spiral bundle (*e*) is markedly reduced


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endorgan degeneration the secondary retrograde neuronal degeneration seems to be very much delayed or incomplete Thus we observed one patient with Alport syndrome who was totally deaf for several years before death and two deaf-mute patients with Usher s syndrome, whose cochleas revealed preservation of a fair number of neurons in spite of severe damage to or ab sence of the organ of Corti (Fig 5*C*) Other cases of profound or total congenital hereditary deafness with very good preservation of cochlear neurons over many years have been reported by Lindsay (1973) and Gacek (1971)

More precise information about this type of hereditary congenital deafness associated with pigment anomalies is provided by histological studies in animals such as deaf mouse mutants (Deol, 1954, Grüneberg, 1956, Ernstson, 1971), the dalmatian dog (Lurie, 1948, Schuknecht et al, 1965, Anderson et al, 1968) and the deaf white cat (Bosher & Hallpike 1965, Mair, 1973) According to the studies of Mair in the deaf white cat, substantial degeneration of the spiral gan glion started only at the end of the first year of life and progressed continuously over the entire observation period of 8 years

Well preserved neuron populations as reported by Schuknecht and Anderson in the dalmatian dog are probably due to the young age (maximum 18 months) at which the animals were sacrificed and examined, before retrograde degeneration had taken place on a larger scale Retrograde degeneration in hereditary cochleo-saccular degeneration certainly pro ceeds much more slowly than it does after destruction of the organ of Corti by acquired factors There is a considerable lag between the complete collapse of the organ of Corti which determines the hearing loss, and the retrograde neuronal degeneration

In the deaf white cat we find during a great part of adult life between 5–10% remaining ganglion cells similar to the situation in retrograde degeneration after experimental destruction of the organ of Corti Even the type of the surviving cells might be the same, as far as can be judged from the available histopathological material Only in the old animal do the surviving neurons also begin to show signs of degenera tion, either due to aging or to the hereditary deafness factors

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Fig 5 (A) C [illegible] 48 [illegible] became

deaf as the consequence of a head blow 60 years ago Total loss of sensory elements and practically total loss of cochlear neurons Partial fibrous obliteration of the perilymphatic spaces (C) Cochlea of a deaf mute patient which d ed at the age of 20 who suffered from Usher s syndrome with a positive family history In spite of an entirely absent organ of Corti in all turns there is a fair number of remaining cochlear neurons (*N*) mainly in the upper turns.

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DISCUSSION

I Friedmann Is regeneration possible? *Wardenburg* syndrome—material on which figures are based

M Lawrence I noticed that the unmyelinated ganglion cells in the normal ganglion section had lobulated nuclei while in the ears in which these unmyelinated ganglion cells were the only cells remaining, the nuclei did not appear lobulated Could there have been changed ganglion cells or was this appearance merely a matter of the particular section shown?

M Portmann You show this degeneration of neurofibres after ototoxic aggression How do you explain the success of cochlear implant in such case? *I saw on your* slides a degeneration of efferent spiral bundle after acoustic trauma Long ago I did the same sort of research and always found an intact spiral bundle after the destruc- [illegible] How do [illegible] rence and Mr Portmann about its importance by the clinical application in cochlear implants Which is the limit in the time of the retrograde degeneration and in which

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CLEARANCE OF MIDDLE EAR EFFUSIONS BY THE MUCOCILIARY SYSTEM

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Abstract There are two extreme types of middle ear effusion leading to hearing loss (*a*) a rubber like effusion seen in secretory otitis media and (*b*) a water like effusion seen in serous otitis media The possibility is considered that the degree of crosslinking in these two extreme cases is the basis of an altered mucus transport rate that leads to an accumulation of effusions and hence impaired hearing.

It has been shown (King et al, 1974) that the requisite rheological property for transport activity is not unique to mucus structural macromolecules but is found with other polymeric systems that are loosely crosslinked e g guaran polyacrylamide, gelatin and agarose Studies on one of these systems, guaran indicate that the transport rate is dependent on the degree of crosslinking with a maximum rate found close to the gel point, i e in a region where there are very few crosslinks per macromolecule

The finding that mucus from different mucociliary epithelial sources involves a chemically similar structural glycoprotein suggests that differences observed in transport rate between various mucus samples are more likely due to differences in crosslinking than chemical variations of the glycoprotein units. By analogy with the model guaran system, it is suggested that the two types of middle ear effusions represent extremes in crosslinking and that their transport rate lie to either side of the optimum presumably represented by the normal secretion Factors such as charge concentration and the influence of temperature on mucus crosslinking are discussed.

Clearance insufficiency of effusions from the middle ear can be considered from the point of inadequacies of the middle ear mucociliary system Theoretically, a ciliated epithelium can malfunction due to various causes and, therefore, be responsible for middle ear clearance deficiency These causes could be an inadequate [illegible] number of cilia, ciliary dysfunction, or an excessive mucus mass A change in mucus characteristics might possibly be considered as well

For decades it was accepted that a ciliated respiratory epithelium lines the Eustachian tube and in the last few years such an epithelium was described as lining important parts of the middle ear as well (Sade, 1966, Lim & Hassel, 1969) This epithelium in the middle ear can function actively in clearing foreign material, as demonstrated experimentally by the rapid clearance of radio-opaque material (Compere, 1958), and as observed directly through tympanic membrane perforations (Sade, 1967)

Normal mucosa of the respiratory tract and the middle ear has a layer of ciliated cells with mucus filled goblet cells scattered among them (Gray, 1928) Below these, there is a stratum of mucus forming glands The ciliary layer is topped by a micron thin mucus blanket (Fig 1), it *is this blanket which moves in response to the* ciliary beat, and carries with it such foreign particles as may be deposited on it These events have been observed in laboratory animals as well as in the middle ear (Sade, 1967)

The ciliated mucosa of the frog and toad palates and of the trachea of the cat are so similar in structure and function to human, including middle ear mucosa, that for convenience it was decided by us to use them for many of our experimental studies (Sade et al, 1970) The universality of the involved principle and its applicability to middle ear problems, while not certain, is highly probable The similarity of the epithelia used and the interchangeability of their mucus has been demonstrated

In our clearance experiments, as already de-

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Fig 1 Histologic section of the mucociliary system specimen is from a toad's palate mucosa The PAS-positive mucus blanket (*arrow*) is seen to come from goblet cells (*G*) Below the mucus blanket cilia are seen (*C*)

monstrated by Hilding (1957), ciliary activity was maintained for many hours even though the preparation was isolated and such preparations can be considered to be representative of *in vivo* conditions It follows that the question as to the precise role of the mucus blanket could be d through experiments with these mod- eparations For this purpose correlations sought between clearance speed and mass various particles, such as charcoal dust, glass and steel balls, and also mercury drops, applied to the active mucociliary epithelial model system described, i e, the isolated palates of the frog and toad or cat trachea (Sade et al, 1970) Such foreign particles were cleared from the preparations at an average speed of 1 cm per minute, the inclination to the vertical at which the preparation was held playing no role Clearance of such foreign materials on each preparation was found to occur at a constant speed in the range from 10^{-7} to 1 g of particle weights tested The upper limit represents the biggest mercury drop which it was possible to handle and the lowest the smallest charcoal dust particle which could still be discerned using an operating microscope (magnification up to ×64) This constant speed apparently reflects the rate of travel of the mucus blanket, the movement of which was observed to be triggered by the particles without being affected by their weight The foreign particles may thus be regarded as passive travellers on top of a biologic conveyer belt

It was also observed that when performing several trials on the same preparation, transport time increased until no clearance of foreign particles was achieved (Fig 2) Cilia, however, continued to beat for hours and at times days after transport had ceased The time from the beginning of the experiment until transport stand still differed greatly according to the type of preparation and the season On preparations where transport of particles had stopped but cilia continued to beat, samples of mucus (accumulated at the cut edge of the preparation) could be placed on the tissue with the result that the mucus blob started moving immediately upon contact with the tissue The velocity attained was, in this case, similar to the initial transport velocity of foreign particles and its velocity did not depend on the load that it carried When mucus samples taken from heterologous epithelia was placed on a depleted preparation, transport was also restored but the velocity obtained was not necessarily the same as with mucus samples from the preparation itself For example, midcycle bovine cervical mu

cus restores transport velocity but the rate of transport attained is only about 30% of that with the frog's own palatal mucus

The fact that solid particles are no longer transported after a certain stage is reached, unless mucus is added, would indicate that the amount of mucus which can be secreted by the isolated system is limited Mucus must be applied externally in order to enable the cilia to bring about transport The condition of the epithelium at this stage is thus a state of mucus depletion

A far reaching conclusion about the role of mucus can be drawn from these experiments Mucus is not only a vehicle for various substances such as immunoglobulins (Gottschalk, 1966), and a moving blanket to which foreign bodies adhere, but it is also an essential mechanical coupler whose presence is imperative for translating ciliary beat into effective clearance Without mucus, clearance does not take place in spite of a regular ciliary beat What then is mucus and what is its structure to make it suit these physiological requirements

Mucus associated with ciliated epithelia is a gel, which in contact with physiological saline does not disperse but remains as a distinct phase The structural components of mucus must thus be intermolecularly crosslinked or at least very heavily entangled with each other Studies performed, using, for example, midcycle cervical mucus which can be obtained in large

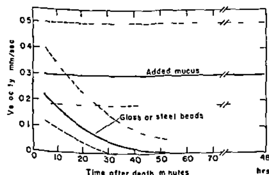


Fig 2 Velocity of transport of foreign particles (curved lower lines) and of added mucus (horizontal) on frog and toad palates as functions of time after death —— Average velocity in 30 experiments - - range of velocities observed

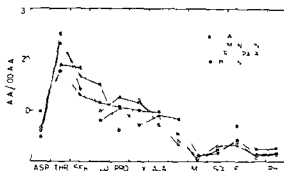


Fig 3 Analyses fo the acidic and neutral amino acids (*a a*) the basic amino acids represent less than 7% of the total amino acids

quantities, indicate that the structural macromolecule is a glycoprotein of high molecular weight ($>10^6$) (Gibbons & Glover, 1959) There are sugar side chains attached via 0-glycosidic links to threosine or serine on the protein backbone A model consistent with the chemical composition indicates that 1 out of every 4–6 amino acids is involved in linkage to sugar The average carbohydrate side chain contains some 9 sugar units, 1 of sialic acid 1 of fucose, 2 galactosamine, 2 of glucosamine and 3 of galactose (Meyer et al, 1973)

A comparison of structural molecules from various mucus sources was performed (unpublished results) The study included mucus samples isolated from patients with secretory otitis media and chronic bronchitis as well as from the cow s cervix at midcycle and the frog's palate In each case the mucus was brought into solution by use of dithiothreitol—a reagent that breaks disulphide bonds (Vered et al, 1972) Fractionation of the solubilized material by centrifugation in a caesium chloride density gradient resulted in the isolation of a glycoprotein fraction containing the bulk of the sugars of the secretion and a fraction containing mainly serum proteins In gel electrophoresis and in sedimentation studies, the glycoprotein fraction behaved as a single entity, though the so-called microheterogeneity in these materials is evident Similarities were noted in the gross chemical composition For example, the glycoproteins could be banded in a caesium chloride density



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applied to the epithelium, the macromolecules penetrate into the serous layer between cilia and interfere with the ciliary beat

In the physiopathology of hearing loss, middle ear effusions are of prime importance (Sade, 1974) and are essentially of two extreme kinds (A) very thick rubber-like effusions, as seen mostly in children and termed "glue ears" secretory otitis media, and (B) water like effusions, as seen mostly in adults and often termed serous otitis media The difference in consistency between the two secretions is very likely the result of a difference in crosslinking (Vered et al, 1972) the water like effusion is probably not a gel On the basis of the evidence presented, these two types of secretions may in fact represent two extremes in the crosslinking of the glycoprotein structural macromolecules In "glue ears the mucus is solid like and presumably heavily crosslinked and in serous otitis, the glycoproteins are not crosslinked at all In analogy with the results in Fig 3, the transport rate may be zero, the two systems being to either side of the optimum represented presumably by the normal secretion In either of these cases the epithelia would be secreting a mucus which could not be transported away fast enough resulting in a net accumulation of secretions in the middle ear which eventually impairs hearing

In the normal state the degree of crosslinking of the glycoprotein in mucus from different sources is probably matched to the required transport rate for the individual tissue In pathological states, such as those of middle ear effusions, crosslinking would seem to be altered The reason for differences in the degree of crosslinking in such glycoprotein based mucus systems is not clear Minor changes in pH and ionic strength of the media have little influence Earlier studies attributed the marked differences in elasticity, a measure of the degree of crosslinking to the sialic acid content of mucus For example, a difference in sialic acid content has been noted for the glycoprotein isolated from the watery midcycle cervical mucus and that from the more elastic secretion found at other stages of the cycle (Gibbons & Glover, 1959, Hatcher & Jeanloz, 1973) However, recently it has been shown that removal of sialic acid with a specific enzyme does not alter the elasticity of mucus (Meyer et al, 1975) nor its biological transportability (King et al, 1974) Differences in crosslinking though may arise as a result of different concentrations of glycoprotein component in the mucus and to the passage of time For example, it has been found that mucus on concentrating does not swell back to its initial volume on standing in buffer This would suggest that additional crosslinks are formed and indeed the material is found to be more rubberlike On the other hand, mucus goes spontaneously into solution on standing The temperature dependence of this process suggests that a relatively low energy of activation is involved indicating disruptions of secondary bonds or complex entanglements (unpublished results)

ZUSAMMENFASSUNG

Es gibt zwei extreme Fälle von Mittelohr Ausfluss die zu einem Gehorverlust fuhren a) ein gummi ähnlicher Ausfluss der bei exsudativer Mittelohrentzundung vorkommt und b) ein wasserahnlicher Ausfluss der bei seroser Mittelohrentzundung vorkommt Es wird die Moglichkeit besprochen dass der Vernetzungsgrad in diesen zwei extremen Fallen die Grundlage der veranderten Transportgeschwindigkeit des Schleimes ist wodurch es zu einer Anreicherung der Sekrete und demzufolge zur Verminderung des Gehores kommt

Es wurde gezeigt (King et al 1974) dass diese notwendige rheologische Eigenschaft fur eine Transportaktivität nicht auf strukturierte Makromolekule im Schleim begrenzt ist sondern auch bei anderen Polymer Systemen gefunden wird die locker vernetzt sind wie z B Guaran Polyacrylamid Gelatine und Agarose Untersuchungen an einem dieser Systeme Guaran zeigen dass die Transportgeschwindigkeit von dem Verretzungsgrad abhängt mit einem Maximum nahe dem Gelierungspunkt das heisst in einem Bereich mit sehr wenigen Vernetzungen pro Makromolekul

Die Beobachtung dass der Schleim von verschiedenen Flimmerschleimhautepithelien eine chemisch ahnliche Glukoproteinstruktur hatte spricht dafur dass die beobachteten Unterschiede der Transportgeschwindigkeit zwischen verschiedenen Schleimproben mehr auf Unterschieden im Vernetzungsgrad beruhen als auf Veränderungen der Glycoprotein Einheiten In Analogie zu dem Guaran Modell wird angenommen dass die zwei Typen von Mittelohr Ausfluss Extreme der Vernetzung darstellen und dass deren Transportgeschwindigkeiten zu beiden Seiten eines Optimums liegen, das bei normaler

Sekretion vorkommt Faktoren der Schleimvernetzung, wie Ladung, Konzentration und der Einfluss der Temperatur werden diskutiert

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DISCUSSION

D Hilding Did you have a chance to test the effect of the mucoid material from a "glue ear" on the cilia of a cat trachea or frog palate?

B McCabe It is clear that this disease is not a pure Eustachian tube disease, but we have all witnessed the phenomenal resolution of symptoms upon simply ventilating the ear I wonder what role you feel that the Eustachian tube does play in this disease

G Zechner Mucus in the middle ear comes from goblet cells, ciliated cells and normal epithelial cells under certain conditions In addition, some mucus comes from the Eustachian tube For ciliary action, I want to give credit to Messerklinger's findings the ciliary organ needs for active transport a gel formation layer within which to beat This gel, which we call mucus, is influenced by pH, ionic concentration and other facts

J Sade (Reply) to Mr *Hilding* You are of course right and this should be done, we have not done it so far because of technical difficulties in transporting the whole lab nearer to the operating theatre and we did not want to carry the mucus to the Weizmann Institute 30 miles away

To Mr *McCabe* The Eustachian tube is obviously not functioning as it should in secretory otitis media i e it probably does not pump air in sufficient amounts into the middle ear but this does not mean that it is mechanically obstructed

To Mr *Zechner* The pH and ionic concentration were indeed measured and their range is rather wide before cilia cease beating

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ELECTROCOCHLEOGRAPHY (ECOG) IN SENSORINEURAL DEAFNESS

J Tyberghein

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Abstract We examined 340 normal ears and cases of sensorineural deafness with electrocochleography using click stimuli (duration 0.5 σ, repetition rate ±10 sec N=1 000 alternately positive and negative analysis time 31 σ) The latency of N_1 is a function of the sound pressure level and of the age of the subject. The intensity of N_1 (in μv) is a function of hearing level and is influenced by the presence of recruitment The pure tone audiogram is a function of the ECOG threshold and of the shape of the reaction obtained at maximal stimulation intensity

In order to check the clinical reliability of electrocochleography, we made, between 1971 and 1973, 340 ECOG in normal individuals and in cases of sensorineural deafness We examined 160 adults and 180 children younger than 10 years with the technique described by Portmann & Aran We passed the electrode through the ear drum in the round window niche under local anaesthesia in adults and under general anaesthesia in children The differential electrode is placed in the concha and the ground electrode on the forehead As acoustic stimulus we use 1 000 clicks of 0 5 σ duration, presented in free field alternately in phase opposition, with a rhythm of ±10/sec The responses were amplified with preamplifiers and filters with an overall frequency response of 0 8-10 000 c/s The reaction analysis time is 31 σ We measure the latency and the intensity of N_1 as a function of stimulus intensity from 20 to 110 dB peak equivalent S P L and control the shape of the reaction obtained as third parameter With the technique we use, an ECOG threshold on 50 dB or better is considered normal and an ECOG threshold on 60 dB or worse as pathological We limit this study to the 199 cases where we obtained an ECOG threshold between 20 and 80 dB S P L In this group we have 137 adults with a very reliable pure tone and Bekesy audiogram

A statistical analysis shows, at the 7 examined frequencies (125, 250, 500, 1 000, 2 000, 4 000 and 8 000 c/s) a very clear-cut difference between the "normal group' (with an ECOG threshold on 50 dB or better, $N=76$) on one side and the 3 pathologial groups on the other side [with an ECOG threshold on 60 dB ($N=21$), 70 dB ($N=13$) and 80 dB ($N=21$)] Within the pathological group the very important pure-tone audiogram threshold dispersion diminishes the diagnostic value of the ECOG threshold But the shape of the reaction obtained at maximal stimulation intensity gives us complementary information on the pure tone audiogram In the pathological group we find two different ECOG types at maximal stimulation intensity (on one side, the peak curve and on the other, the flat curve) which we call, respectively, ECOG type I and ECOG type II (Fig 1) In our pathological group ($N=55$) with an ECOG threshold on 60, 70 or 80 dB we have 32 type I (peak curve) and 23 type II (flat curve) These are 55 adults from whom we have a reliable pure tone and Bekesy audiogram The ECOG type II is very seldom seen in children younger than 10 years we only met it 3 times in the 28 cases with an ECOG threshold between 60 and 80 dB The average pure tone audiogram in the pathological group is worse with an ECOG of

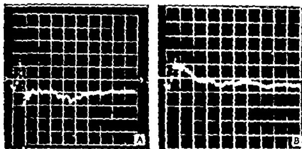


Fig 1 Shape of the reaction obtained at maximal stimulation intensity (110 dB) Analysis time 31 σ A Type I ECOG Peak curve B Type II ECOG Flat curve

type II than with an ECOG of type I The difference between these two average audiograms is significant, with the exception of the 4 000 c/s point (Fig 2) Furthermore, we find a very close relationship in this pathological group between the ECOG type and the Bekesy type An ECOG type I corresponds in 96 % of the cases to a Bekesy type I audiogram, whereas we find in 65 % of type II ECOG, a Bekesy type II audiogram

The type II ECOG is most probably a symptom of recruitment, as is shown by the intensity function of our 86 pathological ears with a type I or II cochleogram (Fig 3) The intensity functions of the normal ears and of the pathological ears with a type I ECOG are identical, whereas the slope of the intensity function of the type II ECOG is much steeper We find a clear cut statistical difference between type I and II from 10 to 40 dB hearing level The external Corti cells are most probably completely destroyed and the threshold is determined by the internal Corti cells with their very steep intensity function

We were able to check this hypothesis in animal experiments Two groups of 8 guinea pigs were injected for 14 days respectively with 150 mg/kg/day Gentamycin and Tobramycin A control group of 8 animals was injected during the same period with isotonic saline Between the 36th and 40th day after the last injection we derived the action potentials with an electrode placed in the round window niche We used the same technique as in our experiments on humans with the 0 5 σ clicks but also with filtered clicks at 16 Kc With unfiltered click stimuli we do not find any difference between the three experimental groups With filtered clicks at 16 Kc we see a threshold shift of 50 to 55 dB for Gentamycin and a steep intensity function that very clearly exceeds the normal values on maximal stimulation intensity It is generally accepted that the ototoxic antibiotics destroy the external Corti cells before they attack the inner ones

The latency of the reaction obtained is the third parameter of the measured action potentials We were able to demonstrate that in normal hearing individuals the latency is not only a function of the S P L but also of the age of the subject For every S P L we find an increase in latency as a function of age Although this difference is greater near threshold, it is still significant from 50 to 110 dB S P L between the 0–10 year group ($N=23$) on the one hand and the 31–40 ($N=28$) and 51–60 ($N=17$) year groups on the other As described by Yoshie, we found that in individuals younger than 50 years and suffering from sensorineural hearing loss, the latency of the AP for a given S P L has a tendency to be longer than in normal individuals of the same age group This tendency is much less important in the oldest group (51–60 years)

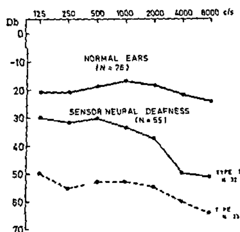


Fig 2 Average pure tone audiogram of the normal ears ($N=76$) with an ECOG threshold on 20 30 40 and 50 dB S P L. Average pure tone audiogram of the pathological ears with an ECOG threshold on 60 70 and 80 dB S P L. and type I ($N=32$) or type II ($N=23$) ECOG.

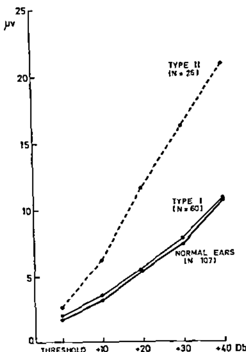


Fig 3 Intensity function of the normal ($N = 107$) and pathological ears ($N = 86$) with type I ($N = 60$) or type II (N 26) ECOG

CONCLUSION

With the technique we use, we consider an ECOG threshold between 20 and 50 dB S P L as being normal When we have an ECOG threshold between 60 and 80 dB we have to take the ECOG type into account The ECOG type I generally corresponds to a high-tone hearing loss without recruitment, whereas the ECOG type II gives a horizontal hearing loss in the pure tone audiogram and is a symptom of recruitment such as we see in Meniere's disease The association of type II ECOG and type II Bekesy audiogram and the steep intensity function of the type II ECOG seem to prove this hypothesis Finally, we could also demonstrate that the age of the subject exerts an influence on the latency of N_1—this must be of some importance in the physiopathology of presbycusis

Much experimental work remains to be done in this field in order to make electrocochleography fully reliable in clinical practice

RÉSUMÉ

Nous rapportons le résultat des examens electrocochléographiques pratiques sur 340 oreilles normales ou atteintes de surdite de perception Nous utilisons comme stimulus 1 000 clics d une duree de 0,5 σ, alternativement positifs et négatifs, à un rythme de ± 10 sec — le temps d analyse est de 31 σ Le temps de latence de N_1 est une fonction de la pression acoustique et de l âge du sujet L'intensite de N_1 (en μv) depend du seuil de réaction electrocochleographique et est influencee par la presence de recrutement.

L'audiogramme tonal liminaire est une fonction du seuil électrocochleographique et de la forme de la reaction obtenue à l intensite stimulatoire maximale.

ZUSAMMENFASSUNG

Wir berichten über die Ergebnisse von 340 Elektrocochleogrammen von normalen Ohren und pathologischen (nur Perzeptionschwerhorigkeiten) Als Stimulus benutzen wir eine Serie von 1 000 Klicks (0,5 σ) abwechselnd positiv und negativ in einem Rhythmus von ± 10/Sek Die Analysezeit betragt 31 σ Dabei ist die Latenz von N_1 eine Funktion des akustischen Drucks und des Alters der Testperson Die Intensitat von N_1 (in μv) hangt von der elektrocochleographischen Schwelle ab und wird durch mogliche Erholung beeinflusst Das Reintonaudiogramm ist eine Funktion der elektrocochleographischen Schwelle und der Reaktionsform auf maximale Stimulationsintensitat.

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DISCUSSION

J Aran I have a comment to make with the correlation between ECoG thresholds and the audiometric thresholds you showed a not too-good correlation between ECoG threshold and 1 000 Hz pure tone threshold But I think that the 1 000 Hz threshold is not enough to characterize the audiogram. When we compared the ECoG threshold with the mean of the audiogram over the six frequencies, then we find a very good correlation We also find the best correlation between ECoG [illegible]

have you also tried other tests of subjective audiometry suggesting the presence of recruitment phenomenon in the type B cases?

J. Tyberghein (Reply) to Mr *Aran* The stimulus I use, being a 0 5 σ click, is quite different from yours (0 1 σ) I think that is the reason why we obtain different results.

To Mr *Bochenek* We only used pure tone and Békésy audiometry in this experimental series

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ELECTROCOCHLEOGRAPHIC STUDY OF A CASE OF LERMOYEZ'S SYNDROME

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Abstract A case of Lermoyez s syndrome was investigated by tone burst electrocochleography in both a period of impaired hearing and in a period of almost normal hearing The changes in threshold values compound action potential waveforms, input-output curves and amplitude latency curves for the compound AP, are described The electrocochleographic data are compared with the psycho acoustic data for this case and with electrocochleographic results in a group of 22 Menière cases Although the symptoms of vertigo and hearing impairment in Lermoyez s syndrome occur in the reverse sequence as compared with the classical Meniere attack the data obtained in this case of Lermoyez s syndrome do not differ substantially from those obtained in a group of Meniere cases

Although it is commonly accepted that Lermoyez's syndrome (Lermoyez, 1929) is a variant of Menière s disease rather than an independent entity (Bailie, 1956 Harrison & Naftalin, 1968, Eckardt & Claussen, 1972), the validity of this statement has been questioned (Eagle, 1948) Comparison of the data obtained by electrocochleography in patients with Lermoyez's syndrome with those from Meniere cases can help to resolve this problem

Lawrence & McCabe (1959) postulated that in Meniere s disease, a rupture of Reissner's membrane is caused by an endolymphatic hydrops the resulting contamination of endolymph with perilymph leading to attacks of vertigo and loss of hearing The clinical symptoms of the vestibular disturbance would subside rather quickly, but the hearing loss would only diminish gradually as the rupture heals and normal sodium and potassium levels are restored The reverse order of the symptoms occurring in Lermoyez's syndrome is not in accordance with this theory

We had an opportunity to perform electrocochleography in a case of Lermoyez's syndrome just after a vertiginous attack, while the hearing was almost normal, and a second time, about 2 months later, just before the next attack of vertigo, when there was considerable hearing loss Fig 1 shows the threshold values at 8 000 Hz and 500 Hz over a period of about 2 years, during which the patient had three series of vertiginous attacks The period in August of 1972 is presented in greater detail, between January 1973 and January 1974 vertigo did not occur The marked changes in the 500 Hz threshold values and the minor changes in the 8 000 Hz threshold values are evident

The patient was an office employee, born in 1933, who for about 6 years had periodically had a sensation of fullness in his left ear lasting for several minutes During the last 2 years he had suffered from a number of vertiginous attacks accompanied by vomiting and sweating Some 10 days before these attacks he noticed the same sensations of fullness but now with tinnitus and hearing loss Immediately after the attacks the tinnitus disappeared and the hearing returned to normal

During such a period of diminished hearing, conventional audiometry showed a perceptive hearing loss at all frequencies Speech audiometry revealed a 45 dB shift without discrimination loss The ABLB test at 500 Hz showed com-

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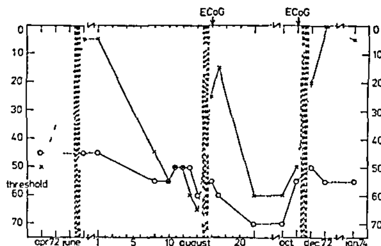


Fig 1 Time course of the subjective threshold values for *500 Hz* (×—×) and *8 000 Hz* (o—o) in a case of Lermoyez s syndrome The periods in which the patient suffered from vertiginous attacks are indicated by the shaded vertical columns Immediately after the attacks the patient s hearing returned to almost normal values for 500 Hz and less or no change occurred at 8 000 Hz. Electr cochleography was performed just after the attack August of 1972 and just before the period of attacks November of 1972 During the almost 2 years since th last attack the hearing has remained normal at all fr quencies except 4 000 and 8 000 Hz.

plete recruitment The tone decay test was negative After the subsequent vertiginous attack, the pure tone hearing threshold values returned to normal

The results of electronystagmography, includ those obtained with caloric stimulation, were normal, except in the vertiginous periods Further ENT, ophthalmologic, neurologic, and general examinations, as well as radiography c the petrous bones and cervical vertebrae showe no abnormalities Laboratory tests of blood cerebrospinal fluid, and urine were within norma limits

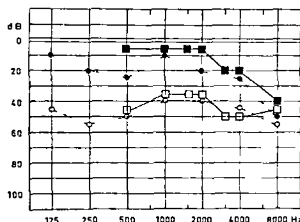


Fig 2 Electrocochleographic threshold values just be

subjective threshold values In general there is good agreement between the two methods with respect to the threshold values

METHOD

Electrocochleography is performed with pure-tone bursts (Eggermont et al, 1974) Unlike click stimuli, this type of stimulus provides frequency specificity up to about 65 dB HL (Eggermont & Odenthal, 1974) The cochlear potentials and whole nerve action potentials (AP) are recorded from the promontory With this experimental setup, threshold values and input output curves of the AP can be determined for the standard audiometric frequencies of 500 Hz and higher

RESULTS

The threshold values obtained with tone burs electrocochleography in this case of Ler moyez s syndrome during a period of impaired hearing as well as during a period of almost undisturbed hearing, are given in Fig 2 together

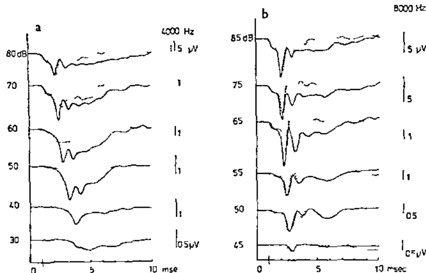


Fig 3a b Compound action potential waveforms for 4 000 and 8 000 Hz tone bursts in both states of hearing. —— APs obtained after the vertiginous attack (good hearing) APs for the period of impaired hearing For 4 000 Hz there is a distinct difference in waveform between the two periods, for 8 000 Hz only minor differences in the compound APs

with the threshold values obtained by conventional audiometry There is fairly good agreement between the electrocochleograms and the subjective audiograms

The compound action potentials obtained at 4 000 and 8 000 Hz are given in Fig 3*a* and *b* The difference in waveform between the two series at 4 000 Hz and the close similarity be-

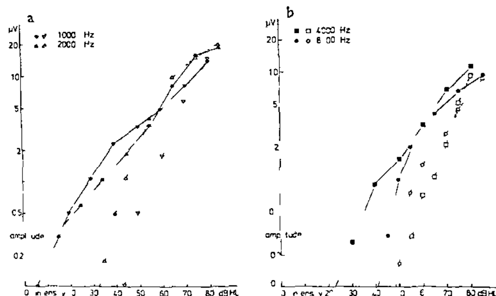


Fig 4a b Input-output curves of the compound action potentials obtained during both states of hearing The solid lines and symbols refer to the normal hearing period the dashed lines and open symbols to the impaired hearing period For 1 000 and 2 000 Hz the threshold difference is 40–30 dB at 80 dB HL the output is the same This results in a steeper input-output curve The same phenomenon is observed at 4 000 Hz, whereas at 8 000 Hz there is only a slight threshold shift and the slopes do not change essentially The increase in slope is thought to reflect the presence of loudness recruitment which was observed during the period of impaired hearing

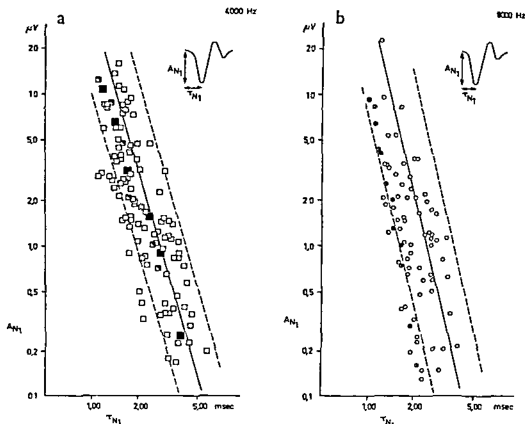


Fig 5a b Amplitude latency data for 4 000 and 8 000 plotted in a scattergram of a large series of Meniere ■ ● Normal hearing state ◪ ◑ impaired hearing state □ ○ the Meniere data —— Regression lines log τ_N on log A_N for normal hearing data the 2σ boundaries for the same group The Lermoyez s data for 4 000 Hz l e fully within the range of the Meniere data (for both hearing states) at 8 000 Hz—where less or no change in the hearing threshold occurred—the values l e near the lower 2σ boundary

tween these waveforms at 8 000 Hz is an indication for a restricted localization of the disturbance and also reflects the frequency specificity of the method

The input-output curves obtained during a period of relatively normal hearing (——) and during a period of hearing loss ( ) are shown in Fig 4*a* and *b* The curves for 1 000 and 2 000 Hz, which are initially similar to those found in normal ears become steeper in the period of hearing loss The 4 000 Hz curve showing a slightly elevated threshold also becomes steeper whereas the 8 000 Hz curves are nearly equal for both periods The steepness of the input output curves of the AP might be related to the psycho-acoustical phenomenon called loudness recruitment (Portmann et al 1973 Schmidt et al 1974)

The amplitude-latency data ($A_N - \tau_N$) for 4 000 and 8 000 Hz obtained in the normal hearing state and during the period of hearing loss do not differ substantially, even at 4 000 Hz where a threshold difference of 30 dB is found In Fig 5*a* and *b* these data are plotted in scattergrams for comparison with data from 22 Meniere patients The solid lines represent the linear regression lines for log τ_N on log A_N for a group of 18 normal ears the dashed lines are the 2 σ boundaries for that group of normal ears Regression lines (not shown) through the 4 000 Hz data obtained before and after the attack nearly coincide and also lie completely within the boundaries of the normal group as do most of the Meniere data The 8 000 Hz data again show a close similarity between both amplitude latency lines (not shown) but now

the points are centered around the lower 2 σ boundary of the normal group corresponding to the boundary of the Meniere data

CONCLUSIONS

The electrocochleographic investigation of this patient with Lermoyez's syndrome showed the following characteristics during the period of hearing loss

(1) Abnormal, steep, input-output curves for the AP

(2) Normal amplitude latency curves, except for 8 000 Hz

These characteristics are compatible with those found in a large group of Meniere patients studied by electrocohleography (Schmidt et al, 1974) The electrocochleographic findings support the conclusion that—at least as far as the cochlea is concerned—this syndrome is closely related to Meniere's disease

RÉSUMÉ

Un cas du syndrome de Lermoyez a ete examiné par électrocochléographie au son bref, aussi bien dans une periode où la fonction auditive est pertubee que dans une periode ou elle est plus ou moins normale

Les variations dans les seuils, les formes du potentiel d'action global, les fonctions d entrée sortie et les relations amplitude latence pour le potentiel d action global sont decrites Les données électrocochleographiques sont comparees avec les donnees de psycho acoustique pour ce cas et avec les résultats electrocochleographiques dans un groupe de 22 cas de maladie Menière. Malgre le fait que les symptômes de vertige et de perturbation auditive dans le syndrome de Lermoyez apparaissent dans l ordre inverse, comme c est le cas avec la crise classique de la maladie Menière, les données obtenues dans ce cas du syndrome de Lermoyez ne different pas substantiellement de ceux obtenus dans un groupe de cas de maladie Menière

ZUSAMMENFASSUNG

Ein Fall vom Lermoyez Syndrom wurde mittels Ton impulsreiz Elektrocochleographie wahrend einer Periode von Horverlust und wahrend einer Periode von nahezu normalem Horen untersucht Die Änderungen der Horschwelle das Summenaktionspotential Reizstarke Erregungskennlinien und Amplitude Latenzzeitkennlinien des Summenaktionspotential sind beschrieben worden

Die elektrocochleographischen Daten sind verglichen worden mit den psychoakustischen Daten dieses Falles und mit den elektrocochleographischen Ergebnissen einer Gruppe von 22 Meniere Patienten

Obwohl Schwindelgefuhl und Horverlust im Lermoyez Syndrom in umgekehrter Folge erscheinen wie im klassischen Meniere Krankheitsbild, unterscheiden die elektrocochleographischen Daten sich nicht wesentlich von denen, die wir gefunden haben bei einer Gruppe von Meniere Patienten

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DISCUSSION

L. B W Jongkees We also treat patients suffering from Menière s disease We forbid them to smoke to drink alcoholic drinks, to look at women, we even operate upon them basing ourselves on theories and hypotheses that have never been proved! Every attempt to obtain a better insight into the basic processes of Menière s disease should be applauded I should like to know what the results of vestibular examination of this patient were

P H Schmidt (Reply) to Mr *Jongkees* Yes vestibular examination was done in this patient The results are in good agreement with those found in Menière patients In connection to this I should mention that some six months later this patient had another attack, this time however, in the "normal" order of Meniere s disease

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ÉTUDE DE LA MINIMISATION d'UNE GRILLE DE MESURE EN AUDIOMETRIE

A H Morgon

Travail du Centre d'Audiophonologie de Lyon, Lyon, France

Le depistage de la surdite est une question interessant non seulement le petit enfant mais aussi l'enfant scolarise, la jeune recrue, l'apprenti, l'ouvrier expose au bruit. Le but du depistage est bien de reveler une atteinte auditive manifestee par une baisse de la courbe audiometrique Le test de depistage doit, dans la mesure du possible, repondre a un certain nombre de criteres efficacite, rapidité Un certain nombre d appareils existent en audiometrie infantile ou en audiometrie conventionnelle, dite d adulte

Pour vérifier le bien fonde du choix des frequences et des niveaux d intensite, une etude a realisee au Centre d'Audiophonologie de

audiogramme classique comporte 66 points mesure definis par 6 frequences de 250 à 8 000 Hz d'octave en octave et de 0 à 100 dB de 10 en 10 dB Cet audiogramme classique realise une grille à 66 points Nous avons pose a un ordinateur la question de la minimisation de cette grille de mesure

Le probleme a ete traite a l aide de la theorie de l information

BASES MATHEMATIQUES

Rappelons que, par definition l incertitude $H(X)$ sera une variable aleatoire discrète X prenant les valeurs $X_1\ X_2 \quad X_n$ avec les probabilités P_1, $P_2 \quad P_n$ est

$$H(X) = -\sum_i p_i \log_2 p_i$$

On va calculer l incertitude sur la connaissance d une courbe dans chaque case de la grille

En effet, dans la case j passent nj courbes differentes, la probabilité de reconnaître la courbe C_i est $p_i = 1/nj$ et l'incertitude sur la reconnaissance des courbes dans cette case est.

$$Hj = -\sum \frac{1}{nj} \log_2 \frac{1}{nj}$$

soit

$$Hj = \log_2 nj$$

Dans la case suivante $j+1$ passent n_{j+1} courbes parmi les nj precedentes $(nj+1 < nk)$ et l'incertitude $H_{j+1} = \log_2 [n_{j+1}]$ diminue, elle doit être nulle dès que l'on peut reconnaitre la courbe

Si toutes les courbes sont reconnaissables, on doit avoir

$$S = \sum_K \sum_J H_{jk} = 0$$

si H_{jk} est l incertitude sur la courbe K dans la case j

Un programme machine modifie les grilles (diminution du nombre des frequences, suppression de niveaux d'intentie) et à chaque nouvelle configuration teste si S est nul Il sort les combinaisons minimales en frequence et en intensité qui verifient cette condition

METHODOLOGIE

Pour traiter cette question, deux méthodes ont ete employees
— la methode des courbes
— la méthode des zones

A) *La Methode des Courbes*

A partir de dossiers cliniques d'enfants sourds, 24 audiogrammes pathologiques les plus cou

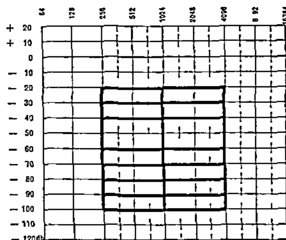


Fig 1 Methode des courbes Contrainte 1

rants definis sur la grille classique ou audiogramme americain ont ete retenus, ils repondent à une statistique de 624 cas de surdite de perception de l'enfant

La minimisation du nombre de points de mesure a ete realisee sous trois contraintes

— contrainte 1 reconnaissance de 24 courbes

— contrainte 2 reconnaissance de certains groupements de courbes

— contrainte 3 reconnaissance de groupements de courbes choisis par les experimentateurs cliniciens

1 — *Contrainte 1*

La contrainte 1 consiste pour l ordinateur a proposer une ou plusieurs grilles permettant de reconnaitre les 24 courbes en les differenciant *formellement chacune*

L'ordinateur propose une grille a 24 points

3 frequences 250 1 000, 4 000

8 niveaux d intensite 20, 30, 40, 60 70 80, 90 et 100 dB

2 — *Contrainte 2*

L ordinateur doit reconnaitre des groupements de courbes Trois solutions sont proposees

Grille I_2 il s'agit d une grille a 12 points avec

— 3 frequences 500 2 000, 8 000

— 4 niveaux d intensite 30, 60, 90, 100 dB

Cette grille permet de reconnaitre 8 groupements de courbes

— Groupe I courbes 1, 2, 3, 4, 5, 6

— Groupe II courbes 7, 8, 10, 11

— Groupe III courbes 9, 21

— Groupe IV courbes 12, 14

— Groupe V courbes 13, 20

— Groupe VI courbes 15, 16, 17, 18

— Groupe VII courbes 19, 22

— Groupe VIII courbes 23, 24

Ces regroupements sont tres mauvais puisqu'ils melangent des courbes de demi surdite et de surdite profonde ainsi qu'ils ne differencient pas entre les courbes ayant une frequence 4 000 ou non

Grille II_2 il s agit d une grille à 9 points avec

— 3 frequences 500, 2 000, 8 000

— 3 niveaux d intensité 30, 90, 100 dB

Cette grille permet de reconnaitre 4 groupes de courbes

— Groupe I courbes 1, 2, 3, 4, 5, 6

— Groupe II courbes 7, 8, 9, 10 ,11, 12, 13, 14, 19, 20, 21, 22

— Groupe III courbes 15, 26, 17, 18

— Groupe IV courbes 23, 24

Les mêmes reproches sont avances que pour la grille I_2

Grille III_2 il s'agit d une grille a 9 points avec

— 3 frequences 500, 2 000, 8 000

— 3 niveaux d intensite 30, 80, 100 db

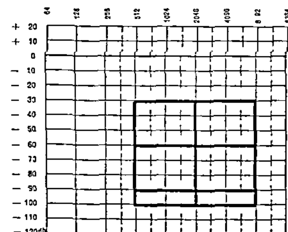


Fig 2 Méthode des courbes Contrainte 2 Solution I_2

Cette grille sépare 8 groupes :
- — Groupe I . courbes, 1, 2, 3, 6
- — Groupe II . courbes 4, 5, 10
- — Groupe III courbes 8, 9, 11
- — Groupe IV . courbes 12, 14
- — Groupe V courbes 15, 16, 17, 18
- — Groupe VI . courbes 19, 22
- — Groupe VII : courbes 7, 13, 20, 21
- — Groupe VIII courbes 23, 24

Dans ce cas encore, les regroupements sont mauvais

3 — Contrainte 3

Les courbes ont ete classées en 7 groupes selon leurs similitudes audiometriques
- — Groupe I · courbes 1, 2, 3, 4, 5, 6
- — Groupe II courbes 7, 12, 13, 21
- — *Groupe III courbes 8, 9, 10, 11, 14, 22*
- — Groupe IV courbes 15, 16, 17
- — Groupe V courbes 18
- — Groupe VI courbes 19, 20
- — Groupe VIII courbes 23, 24

Une solution est retenue par l'ordinateur une grille a 12 points avec
- — 3 frequences 500, 2 000 et 8 000
- — 4 niveaux d intensite 30, 50, 80 et 100

La grille est peu differente de la premiere solution en contrainte 2

La critique qui peut être formulée est le nombre eleve de points, il est pourtant tres intéressant d'observer les frequences et les niveaux d'intensité choisis par l'ordinateur

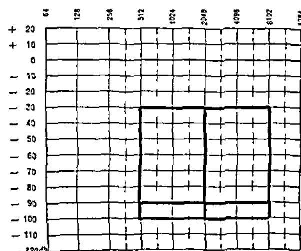


Fig 3 Méthode des courbes Contrainte 2. Solution II_1

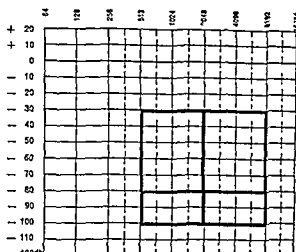


Fig 4 Methode des courbes. Contrainte 2. Solution III_1.

Les frequences retenues sont les fréquences 500 et 2 000 qui sont les fréquences conversationnelles

Quant aux niveaux d'intensité, ils se situent dans deux zones 30, 50 dB et 80, 100 dB La ligne des 90 dB separe bien dans la réalite les surdites profondes des demi surdites

B) *Methodes des Zones*

L'étude a eté generalisee en prenant non plus en compte 7 groupements des 24 courbes initiales mais en determinant 11 zones dans la grille, toutes *les courbes interieures a une zone étant* supposée être d'un même type Chaque zone est definie par une courbe limite supérieure et une courbe limite inferieure

La minimisation de la grille a eté conduite de la façon suivante on constitue un tableau ayant en colonne les frequences (6 pour la grille classique) et en ligne toutes les combinaisons 2 à 2 des 11 zones soit $C_{11}^2 = 55$ lignes Chaque case du tableau contient un 1 ou un 0 suivant que les 2 zones relatives a la combinaison sont distinguables ou non On cherche la ou les associations minimales des fréquences entraınant des sous tableaux qui conserveront au moins un 1 *par ligne Ensuite, pour chacune de ces* associations, on va chercher le nombre minimum

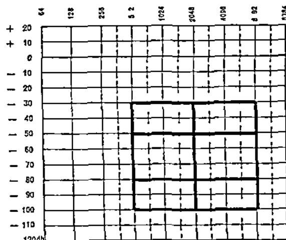


Fig 5 Methode des courbes Contrainte 3

de niveaux d intensite qui entrainera un tableau répondant à la condition precedente (au moins un 1 dans chaque ligne)

Cette analyse conduit a une grille a 21 points avec

— 3 frequences 250, 2 000, 8 000

— 7 niveaux d intensite 10, 30, 40, 50 60, 70 et 100 dB

La solution de cette grille a 21 points ramene pratiquement a celle de la grille proposee dans la contrainte 1 Le nombre eleve de points est en fait contraire a l esprit du depistage qui vise à detecter une surdite et non une courbe audiometrique

Aussi, les zones ont-elles ete simplifiees et 4 zones seulement ont ete retenues

— une zone 1 d audition normale

— une zone 2 de demi surdite

— une zone 3 de demi surdité profonde

— une zone 4 de surdite profonde

On considere que la frontiere (horizontale) entre 2 zones appartient uniquement a la zone superieur ce qui est justifie par la definition même des niveaux d intensite

La methode des zones simplifiee conduit a une grille a 6 points avec

— 3 niveaux d intensite 30, 70 et 100 dB

— 2 frequences F_1 et F_2 a choisir parmi 9 combinaisons

F_1	F_2
250	2 000
250	4 000
250	8 000
500	2 000
500	4 000
500	8 000
1 000	2 000
1 000	4 000
1 000	8 000

Le choix de la frequence F_1 fait intervenir des donnees cliniques et les types de courbes habituellement rencontres il est plus aise de supposer le niveau de la frequence 250 a partir de

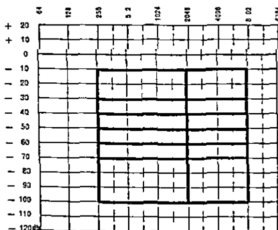


Fig 6 Methode des zones

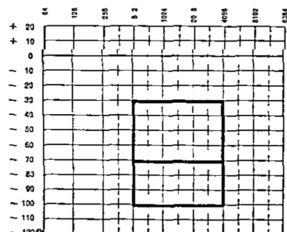


Fig 7 Methode des zones simplifiee

la frequence 550 que le contraire Quant au choix de la fréquence 1 000 il pourrait etre bon si les deux premiers niveaux d intensite etaient plus rapprochés mais la différence de 30 à 70 dB est trop importante

Choix de la fréquence F_2 la frequence 8 000 est souvent difficile a tester Les ecarts entre les frequences 4 000 et 2 000 sont souvent im portants

Les 2 points frequentiels donnent une idee de la pente de la courbe et les points d intensite une idee de la profoundeur du déficit

On est conduit a proposer une grille a 6 points avec
— 3 niveaux d intensité 30 70 et 100 dB
— 2 frequences 500 et 4 000

CONCLUSION

Un appareil simple permet avec 6 points de situer une surdité L exploration recoupe les donnees de l exploration clinique avec l audi tory screening test dont les fréquences s eche lonnent entre 500 et 4 000 et dont les niveaux d intensite se placent exactement dans les deux zones d intensité moyenne et d intensité forte

Cette approche est proposee en vue d une simplification du depistage de l audition Elle peut s appliquer a l adulte Dans tous les cas ou les reponses se situent au dessous de la ligne 30 dB sur les deux frequences retenues 500 et 4 000 un examen audiometrique spécialisé s avere necessaire

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DAS VERHALTEN AKUSTISCH VORGESCHÄDIGTER BIOPOTENTIALE BEIM MEERSCHWEINCHEN NACH BESCHALLUNG MIT WEISSEM RAUSCHEN

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Abstract 62 Meerschweinchen wurden zweimal 40 Minuten mit einem Breitbandrauschen von 20–20 000 Hz, 105 dB SPL beschallt Zwischen beiden Schallbelastungen lag eine Pause von 24 Stunden Abgeleitet wurden die CM, die NAP und SEP Der Schadigungsgrad der akustischen Biopotentiale nach zweimaliger Beschallung war unterschiedlich Wahrend die CM nur wenig betroffen waren, zeigten die NAP eine deutliche Beeinträchtigung der Erregung und Adaptation und die SEP eine Einschränkung vor allem der Erregung Daraus ist zu schließen, daß die CM allein nicht als Kriterium fur eine Schadigung des akustischen Systems durch Schalltraumata ausreichen Weiter ergibt sich, daß dieses System in der Lage ist kurzfristig periphere Schaden zentral auszugleichen und daß eine erneute Belastung nicht zwangsläufig zu einer Addition oder Kumulation der Schadigung fuhren muß

Die Frage nach dem Verhalten einer vorgeschädigten Cochlea bei erneuter Belastung gewinnt heute bei Zunahme des Umweltlärms immer mehr an Bedeutung Morphologische Untersuchungen sind dabei nach unserer Meinung nur bedingt aussagekräftig, denn im Lichtmikroskop fiel uns immer wieder die Einförmigkeit der morphologischen Reaktionen an den Strukturen der Cochlea, unabhängig von der Reizart, auf (Beck & Michler, 1960) Als Beispiel dieser Gleichförmigkeit sind auf Abb 1 die Schwellkerne der äußeren Haarzellen nach Einwirkung verschiedener Reize (Sinustonbeschallung, Sauerstoffmangel, Hochfrequenzstrom, Ultraschall) zusammengestellt

Im Gegensatz hierzu durften nach unserer Erfahrung die elektrophysiologischen Daten bei Ableitung der akustischen Biopotentiale eine differenziertere Aussagekraft besitzen So lassen sich z. B bei unterschiedlicher Gabe von Streptomycin differente Schädigungsmuster peripherer und zentraler Reizantworten aufzeigen (Stange et al, 1964, Theissing, 1963) Noch deutlicher wird ein solcher Unterschied beim Vergleich der Einwirkung von Neomycin- und Gentamycinsulfat (Stange et al, 1968, Soda et al, 1968) Daneben sind Funktionsstörungen der Cochlea mit Hilfe der Ableitung akustischer Biopotentiale wesentlich früher nachweisbar als durch morphologische Reaktionen (Stange et al, 1966)

Ausgehend von diesen Überlegungen führten wir in den letzten Jahren Experimente zum akustischen Streß mit Ableitung der akustischen Biopotentiale durch Dabei bestätigte sich einmal die bereits morphologisch aufgezeigte Tatsache der Frequenzabhängigkeit der Lokalisation einer Schädigung (Benning, 1973) Weiter konnten wir feststellen (Benning & Stange, 1972), daß die cochlear microphonics allein nicht als Kriterium für eine Schädigung des akustischen Systems durch Schalltraumata ausreichen. Aus diesem Grund bezogen wir in der Folge auch die Ableitung des Nervenaktionspotentials und des langsamen Rindenpontentials in unsere Experimente mit ein (Benning, 1973) Die Ergebnisse dieser Versuche ließen ein unterschiedliches Verhalten der einzelnen Biopotentialen erkennen So treten z B peripher nachweisbare Schaden zentral nur zum Teil in Erscheinung, dies besonders in adaptiertem Zustand

In Fortführung dieser Untersuchungen stellten wir uns folgende Fragen, die sich im wesentlichen aus dem zunehmend wichtiger werdenden

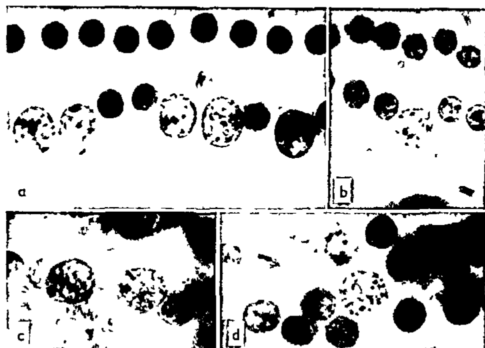


Abb 1 Schwellkerne nach verschiedenen Reizen (*a*) Sinuston, (*b*) Sauerstoffmangel (*c*) Ultraschall, (*d*) Hochfrequenzstrom (Aus Beck Chl u H H Michler 1960 Arch Ohr Nas u Kehlk Heilk *174* 496)

Problem der Lärmbelastung bzw der Lärmschwerhörigkeit ergeben

1 Wie verhält sich ein akustisch belastetes Ohr nach erneuter Beschallung?
Wieweit tritt eine Erholung von der ersten Schallbelastung nach einer Pause von 24 Stunden ein?
3 Welche Reaktionen zeigen die Biopotentiale auf einen nach 24 Stunden einsetzenden zweiten Schall Streß?

METHODIK

62 Meerschweinchen wurden mit einem Breitbandrauschen von 20–20 000 Hz, 105 dB SPL, 40 min lang beschallt und die cochlear microphonics (CM) und das Nervenaktionspotential des Hornerven (NAP) am hinteren Wall des runden Fensters abgeleitet Die Elektrodenlage richtete sich nach dem experimentell ermittelten Ort der größten Amplituden Die Ableitung der langsamen Rindenpotentiale (SEP) erfolgte contralateral der Beschallung an der akustischen Area AI Der verwendete Kopfhörer strahlte die Frequenzen 20–5 000 Hz mit 105 dB Intensität ab, die höheren Frequenzen mit um 40–50 dB verringerter Lautstärke Die Ableitungsdauer betrug 160 min Nach 24 Stunden Pause erneute Registrierung der Biopotentiale und anschließend Beschallung unter den gleichen Bedingungen

ERGEBNISSE

Während der ersten Schallbelastung zeigte sich eine Verminderung der cochlear *microphonics* aller Frequenzen (die verschiedenen Testfrequenzen sind durch unterschiedliche Strichführung gekennzeichnet — Abb 2) unmittelbar nach Beschallungsbeginn mit besonderer Betonung der Frequenz 1 000 Hz Bis zum Ende der Beschallung waren die einzelnen Frequenzamplituden um 30 % bei den hohen und um 76 % bei den niedrigen Frequenzen vermindert Nach 160 min, am Ende des ersten Versuchsabschnitts betrug die Schädigung der CM für die niedrigen Frequenzen nur noch 40 % Innerhalb dieses Zeitraums war also bereits eine deutliche Erholung eingetreten Zu Beginn des 2 Versuchsabschnitts, 24 Stunden später, zeigte sich eine fast völlige Erholung über alle Frequenzen

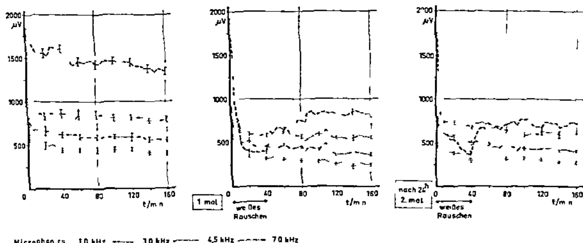


Abb 2 Cochlear microphonics der Frequenzen 1, 0, 3, 0, 4, 5 und 7 0 Khz, durch verschiedene Strichführung gekennzeichnet Die Potentialhöhe in Micro-Volt ist über der Zeit aufgetragen Im linken Bildteil die Normal werte, im mittleren Bildteil die Werte nach einmaligem Weißen Rauschen von 40 Minuten, im rechten Bildteil der zweite Versuchsteil nach 24 Stunden Die Potentiale von 1,0 Khz sind am meisten beeinflußt und zeigen als einzige in beiden Versuchsteilen deutliche Erholung

Nach erneuter Belastung mit Breitbandrauschen war am Ende der Beschallung bei 1 000 Hz eine um 100 µV größere Potentialminderung zu verzeichnen und auch die Erholung bei dieser Frequenz war um 150 µV geringer Die übrigen Frequenzen verhielten sich wie nach der ersten Beschallung Eine wesentliche zusätzliche Schädigung der CM durch das zweite akustische Trauma war also nicht erfolgt

Die Registrierung des Nervenaktionspotentials, aufgezeichnet in gleicher Weise wie die CM (Abb 3), zeigt bei Einsetzen der Beschallung eine deutliche Verminderung der Reizantworten Sowohl Erregung (1/sec) als auch Adaptation (50/sec) sind betroffen Die Amplituden vermindern sich, verglichen mit dem Ausgangswert bis zum Ende der ersten Schallbelastung, um 50 % Danach verlaufen die Kurven weitgehend konstant Als weiteres Zeichen einer Schädigung besteht eine Verminderung der Erregungs Adaptation, die sich nach Aussetzen der Beschallung nur wenig erholt Nach 24 Stunden Pause ist im Gegensatz zu den CM keine Erholung zur Norm eingetreten Die Er-

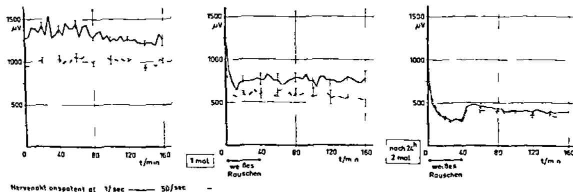


Abb 3 Nervenaktionspotentiale des Hornerven Gleiche grafische Darstellung wie Abb 1 Erregung und Adaptation sind durch verschiedene Strichführung gekennzeichnet. Die zweite Beschallung nach 24 Stunden hat einen Additionseffekt auf die Minderung der Horpotentiale Ferner ist die Erregungs Adaptation beinahe aufgehoben.

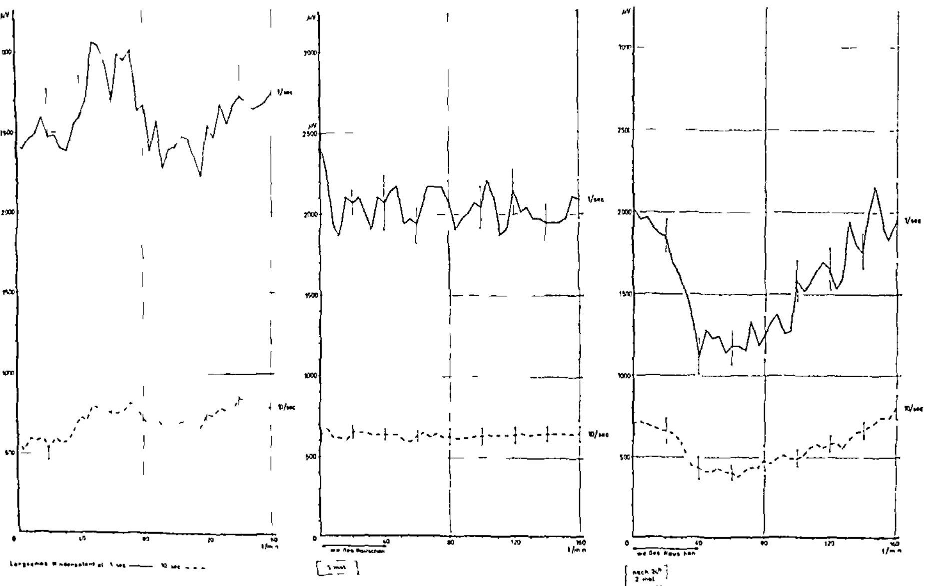

μV
1/sec
10/sec
1/min
2500
2000
1500
1000
500
0
40
80
120
160
nach 24h
2 mal

Abb. 4

regung bleibt um 20% gegenuber dem Ausgangswert vermindert Diese Reaktion ist als Ausdruck einer großeren Beeinflußbarkeit des Erregungsstoffwechsels gegenüber dem Adaptationsstoffwechsel zu deuten Die erneute Schallbelastung fuhrt zu einem weiteren Absinken von Erregung und Adaptation, wobei auffallt, daß die Erregungs Adaptation fast völlig aufgehoben ist Bis zum Ende des zweiten Versuchsabschnitts erholen sich die Potentiale nicht, so daß, gemessen am Ausgangswert, eine Amplitudenminderung von 60% für Erregung und Adaptation zuruckbleibt Im Gegensatz zu den CM zeigt die zweite Schallbelastung beim NAP eine deutliche zusätzliche Schädigung

Das langsam evozierte Rindenpotential (Abb 4) laßt nach einmaliger Beschallung bei der Erregung (1/sec) eine Potentialminderung von 20% erkennen, während bei der Adaptation (10/sec) kein signifikantes Absinken der Potentiale festzustellen ist Die erneute Beschallung hat eine deutliche Minderung der Erregungswerte zur Folge Nach Absetzen der Beschallung tritt, etwa 80 min nach Versuchbeginn, eine Erholung ein, die jedoch den Ausgangswert nicht wieder erreicht Ein der Erregung ähnlicher Kurvenverlauf ist fur die Adaptation zu verzeichnen Auch hier beginnt die Erholung 40 min nach Schallende und setzt sich kontinuierlich bis zum Versuchsende fort Hier aber ist im Gegensatz zur Erregung der Ausgangswert wieder erreicht

DISKUSSION

Den von uns abgeleiteten akustischen Biopotentialen (CM, NAP, SEP) gemeinsam ist die weitgehende Erholung in der 24-stündigen Versuchspause Gemeinsam ist auch ihr Verhalten nach der zweiten Schalleinwirkung, das ähnlich wie beim ersten akustischen Streß abläuft Nur macht sich jetzt die Vorschädigung durch ein Absinken der Potentiale unter die Potentialhöhe im ersten Versuchsabschnitt bemerkbar

Deutlich unterschiedliche Reaktionen zeigen sich jedoch bei Betrachtung des Schädigungsgrades der einzelnen Potentiale nach Lärmbelastung mit Breitbandrauschen Bei den CM reagiert die Frequenz 1 000 Hz mit dem größten Potentialabfall Diese Tatsache deckt sich mit den experimentellen Ergebnissen von Benning (1973), nach denen sich bei spezifischer Schallschadigung die microphonics bei dieser Frequenz am empfindlichsten zeigen Die CM bei 1 000 Hz erholen sich innerhalb der Versuchspause Bei erneuter Beschallung reagiert dann ausschließlich die Frequenz 1 000 Hz mit einem Potentialabfall Allerdings zeigt sich am Versuchsende, daß die CM insgesamt nur gering geschädigt werden

Ein anderes Verhalten zeigen die NAP Hier ist eine deutliche, bis zum Versuchsende anhaltende Schadigung fur Erregung und Adaptation zu erkennen, sichtbar am Absinken der Potentiale und der Verkleinerung der Erregungs-Adaptation Im Gegensatz zu den CM ist also bei den NAP durch die zweite Beschallung eine weitere Schädigung festzustellen

Auch beim SEP sind Schädigungen von Erregung und Adaptation nachweisbar Allerdings ist die Erregung wesentlich starker betroffen als die Adaptation, die sich am Versuchsende wieder voll erholt hat Diese Tatsache ist am ehesten dadurch zu erklären, daß der Stoffwechsel im Bereich der primären Sinnezellen bzw der Erregungsstoffwechsel im zentralen Abschnitt einer großeren Beeinflußbarkeit unterliegt als der Adaptationsstoffwechsel Fur das Horen, das normalerweise im akustisch adaptierten Zustand stattfindet, kann dies bedeuten, daß das akustische System in der Lage ist, kurzfristig peripher vorhandene Schaden zentral auszugleichen

Die Erholung der Adaptation der SEP macht weiter wahrscheinlich, daß nur nach sehr lang- und intensiver Schalleinwirkung au

Abb 4 Langsames Rindenpotential Erregung und Adaptation durch verschiedene Strichfuhrung gekennzeichnet Gleiche grafische Darstellung wie Abb 1 Hier wird eine *besondere Beeinflussung der Erregung deutlich* die im zweiten Versuchsabschnitt in Gegensatz zum ersten eine Erholung nach 80 Minuten zeigt Die Adaptation ist bei der ersten Beschallung kaum, bei der zweiten Beschallung signifikant beeintrachtigt. Es erfolgt jedoch auch hier wie bei der Erregung, eine Erholung bis zum Versuchsende wobei der normale Ausgangswert wieder erreicht wird

in der Adaptation eine permanente Hörschädigung subjektiv merkbar wird, wobei das akustische Sinnessystem sicher lange Zeit deutlich überschwellige Reize unverändert wahrzunehmen in der Lage ist Bei erneuter Belastung muß es also nicht zwangslaufig zu einer Addition oder Kumulation von Potentialschädigungen kommen, wobei jedoch sicher Art und Dauer der Noxe eine entscheidende Rolle beim Grad der Schädigung spielen

Entsprechende Untersuchungen geschadigter akustischer Biopotentiale unterschiedlicher Hörbahnabschnitte in Bezug auf Kennlinie und Schwelle finden sich bei Benning & Stange (1971)

Da mit Hilfe der evoked response audiometry (ERA) unblutig die Hirnrindenpotentiale abgeleitet werden, sind unsere Versuchsergebnisse auch für die Klinik von Bedeutung Sie machen wahrscheinlich, daß mittels der ERA erst spät Schäden erfaßt werden Zur Früherfassung peripher gelegener, durch Schalltrauma verursachter Läsionen, ist eher die Electro-Cochleographie (ECOG) geeignet Mit ihr könnte u U eine frühe Aufdeckung von Berufs- und Umweltschäden möglich sein

Andererseits weisen diese Ergebnisse darauf daß die ECOG allein nicht ausreicht, um diagnostisch Horstörungen und damit subempfundene Horeinschränkungen festzulegen Dieses Problem stellt sich besonders bei der Erfassung der kindlichen Schwerhörigkeit Hierbei ist nach unseren Erfahrungen und denen anderer Autoren wichtig, Kennlinienverlauf einiger Frequenzen und Latenzverhalten fur N_1 der zentralen Potentiale zu ermitteln Besonders letzteres ist von Bedeutung da N_1 fur beide Ohren im pathologischen Fall verschieden sein kann, was zu erheblichen Schwierigkeiten bei der Hörgeräteversorgung führt, deren Ursache oft ohne Kenntnis der Latenz nicht gefunden wird (Benning et al , 1972 Holm & Stange, 1972, 1973, Stange, 1972, 1973, 1974)

SUMMARY

62 guinea-pigs were twice exposed to white noise 105 dB SPL, 20 to 20 000 cps for 40 minutes with a break of 24 hours in between. The cochlear microphonics (CM) the compound action potentials of the acoustic nerve (NAP) and the slow evoked potentials (SEP) were recorded. There were different degrees of damage to be seen in the acoustic biopotentials. The CM showed little damages only whereas the compound action potentials of the hearing nerve showed significant impairments in excitation and in adaptation. In the SEP excitation was decreased From this we may conclude that the CM must not be the only criterion for judging damages in the acoustic system after acoustic trauma Furthermore we see that the central acoustic pathway is capable to compensate peripheric hearing damages within a short time An additional exposure to noise does not necessarily add or cumulate the degrees of damage

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DISCUSSION

H Spoendlin The observation that the action potential is more affected by acoustic trauma than the cochlear microphonics, confirms our findings of early damage to the afferent dendrites to the inner hair cells

C R Pfalz Worauf beruht die zentrale Kompensation des peripheren Ausfalles?

C Beck (Antwort) zu Mr *Spoendlin* Die stärkere Schadigung der NAP gegenüber den CM ist sicher dadurch zu erklaren, dass die entscheidende Stoffwechselstörung an den Nervendigungen, also an der 1 Synapse stattfindet

Zu Mr *Pfalz* 3 Faktoren sind von Bedeutung Die Reaktion der SEP auf die akustische Belastung ist besonders deutlich bei der Erregung Dies besagt, daß der Adaptationsstoffwechsel weniger empfindlich ist als der Erregungsstoffwechsel Da das Horen normalerweise in adaptiertem Zustand stattfindet, so werden periphere Storungen erst nach langerer Belastung subjektiv merkbar Daneben ist im uberschwelligen Bereich bei der SEP im Gegensatz zu den NAP ein Recruitment registrierbar Weiter ist an eine Steuerung uber Synapsen- und Regelsysteme zu denken

Acta Otolaryngol 79. 304–309, 1975

RESTING POTENTIALS IN THE INNER SULCUS AND TUNNEL OF CORTI

M Lawrence, Ph D

From the Kresge Hearing Research Institute, Department of Otorhinolaryngology, University of Michigan Ann Arbor, Mich , USA

Abstract Under constant visual guidance and closed circuit television monitoring, electrodes are inserted into the fluid spaces of the organ of Corti The resting electrical potentials are determined with respect to the neutral voltage of the guinea pig and related to other potentials of the auditory labyrinth. The resting potential of the subtectorial space inner sulcus, tunnel and other spaces of Corti's organ are found to be the same as, or slightly more negative than the potential of perilymph. The tectorial membrane isolates the reticular lamina from endolymph which fact, when considered along with other evidence indicates a more biophysical than mechanical role for the membrane A motion picture is presented showing the cells and spaces of the organ of Corti and the placement of electrodes.

In an embryo of about 8 weeks the condensed mesenchyme that surrounds the invaginated otic sicle begins to show two distinct regions, one , immediately surrounding the now fairly- ll delineated membranous labyrinth, remains bout the same in appearance, while the outer part begins to become denser The inner portion then begins to dedifferentiate into a loose reticulum occurring first in the region of what is destined to become the perilymphatic vestibule and scalae of the basal turn of the cochlea This occurs somewhat later in the region of the canals but the differentiation of the perilymphatic labyrinth is practically complete by the twenty-first week

This process continues, with the reticulum increasing in area by progressive dedifferentiation while the more remote precartilage develops into cartilage and then bone of the otic capsule The reticulum immediately surrounding the developing ectodermal membranous labyrinth finally differentiates into three regions There is the *membrana propria* that has condensed into tissue surrounding the membranous labyrinth, outside this membrane are the spaces and tissue that constitute the perilymphatic labyrinth, and the outerpart becomes the endosteum and endosteal membrane

The mesenchymal epithelium (*membrana propria*) fuses with the ectodermal basement membrane of the membranous labyrinth to form the two-cell thick membrane of Reissner anteriorly, and with the posterior ectodermal basement membrane of the *scala media* to form the basilar membrane Figure 1 is a photomicrograph c the scala media of a 15-week fetus showing th joining of these membranous layers

This basilar membrane extends from the osseous spiral lamina to the spiral ligament intc which fibers pass as far as the area of the spira ligament beneath the *stria vascularis* The membrane is a spiral partition which anatomicall separates the *scala tympani* from the organ o Corti lying on the *scala media* surface as par of the ectodermal labyrinth

There are two distinct zones to this basila membrane which we have discussed at length before (Lawrence, 1971) the thin *zona arcuata* extending from the *osseous spiral lamina* to beneath the outer pillar cell and the thicker *zona pectinata* extending from the outer pillar cell to the spiral ligament

Between these membranous boundaries, Reissner's membrane and the basilar membrane are several fluid spaces The largest of these is the

This work was supported by PHS grants NS 11731 and NS-05785 and by funds from The John A Hartford Foundation.

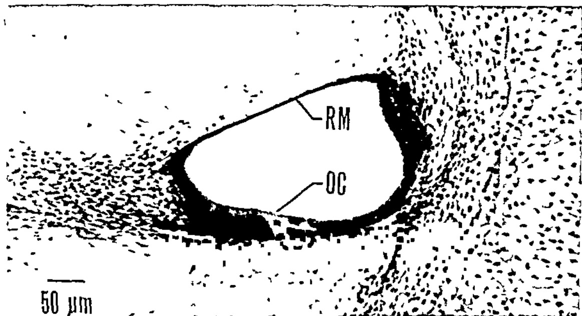


Fig. 1. Formation of the fluid spaces within the scala media of a 14-week human fetus. Perilympatic surfaces of both Reissner's membrane are laid down by mesenchyme to form the *membrana propria*. Fluid spaces with in the organ of Corti are formed by differentiation within the ectodermal neuroepithelium. *RM*, Reissner's membrane. *OC*, organ of Corti. HTB24.

scala media known to contain endolymph which possesses a unique ionic composition and positive electrical potential.

The largest space within the organ of Corti is the tunnel located at the region of the *zona arcuata* of the basilar membrane. The tunnel is bounded by the pillar cells (the rods of Corti) forming a triangle resting upon the basilar membrane.

Other fluid spaces of the organ of Corti are the space of Nuel, spaces between the hair cells, the subtectorial space and the inner sulcus. For some time the question has been raised as to the nature of the fluid within these spaces and as to the boundaries. Of particular interest is the question of whether the subtectorial and inner sulcus fluids are endolymph or part of the organ of Corti fluid space system.

The first suggestion, that the fluid contents of the other organ of Corti spaces could not be endolymph was made by Tasaki et al. (1954) who reasoned that if the unmyelinated nerve fibers coming from the hair cells were immersed in a fluid of high potassium content such as endolymph, the fibers could not conduct impulses, they concluded that the fluid must, therefore, be perilymph.

Engström (1960) pointed out that, until a free communication between this fluid and either endolymph or perilymph was observed, or, until its chemical composition could be demonstrated to be the same as these other two fluids, the fluid must be regarded as a third fluid and he suggested the name *cortilymph*. He based this conclusion mainly on the fact that the organ of Corti is ectodermal in origin whereas the perilymph is of mesodermal origin as already described. The possibility that this must be a third fluid hadal ready been suggested by Borghesan (1950) and Citron et al. (1956). Rauch (1964) has also suggested the possibility of a fourth fluid existing between the tectorial membrane and the reticular lamina which he called the subtectorial lymph.

Electrical Potentials in Fluid Spaces

One way to get some indication of the nature of the fluids in the intercellular spaces is to re-

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There are two distinct zones to this basilar membrane which we have discussed at length before (Lawrence, 1971) the thin *zona arcuata* extending from the *osseous spiral lamina* to beneath the outer pillar cell and the thicker *zona pectinata* extending from the outer pillar cell to the spiral ligament

Between these membranous boundaries, Reissner s membrane and the basilar membrane are several fluid spaces The largest of these is the

This work was supported by PHS grants NS-11731 and NS-05785 and by funds from The John A Hartford Foundation

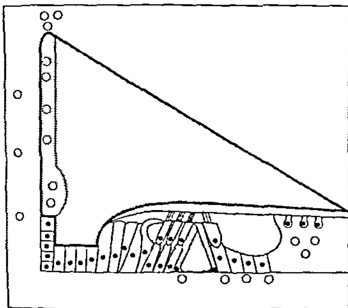


Fig 3 Boundaries of the fluid spaces within the organ of Corti The subtectorial space, inner sulcus, tunnel and Nuel s space have nearly the same electrical potential as perilymph of the scala tympani. The tectorial membrane acts as a barrier protecting the organ of Corti from endolymph. The open circles represent the capillaries in separate areas.

from the negative of the Claudius' cells to the positive potential of the endolymph

Assuming that the highly-positive endolymph bathes the cuticular surface of the hair cell, Davis (1957) proposed that a current flow between this positive and the intracellular negative is modulated by variations in the resistance path produced by movements of the hair cell However, he did have reservations, pointing out that it is difficult to distinguish between a change in resistance to current flow and a change in a generated voltage

If Bekesy's observations are correct then it would appear that the tectorial membrane may play a more important part in the biophysical activities of the organ of Corti than merely a devise for mechanically pulling on the hairs of the hair cells

So it becomes essential in the correct interpretation of these biophysical properties to determine the nature of the fluids within the spaces of the organ of Corti including the sub-tectorial space and inner sulcus

Because of the delicacy of the tectorial membrane, electrode placement must be done with great care Our method, described elsewhere (Lawrence & Nuttall, 1972) and shown here in a motion picture, has been to record the electrical potential of the fluids by a microelectrode passed through the tunnel of Corti, cells, and spaces while under direct visual observation From these experiments (Lawrence et al , 1974) it was demonstrated that the fluid in the tunnel and spaces of the organ of Corti has an electrical potential at, or slightly more negative than that of perilymph of the scala tympani, and the large negative potentials encountered are intracellular

Fluid of Subtectorial Space is Not Endolymph

The tectorial membrane is sealed (Fig 3) against endolymph with the subtectorial and inner sulcus fluids at the same electrical potential as the fluid of the tunnel It has been suggested by some that, because the tectorial membrane is not a cellular membrane, maintaining a highly positive endolymph on one side is inconceivable But it must be kept in mind that endolymph is an extracellular fluid occupying a space surrounded by many different kinds of very active cells and all that is required of the tectorial membrane is that it be a dielectric no different in insulating properties than those portions of a cell membrane between the pores permitting ion transport

That the tectorial membrane is sealed to Hensen's cells is supported by early dissections in

which the membrane was observed in its natural, chemically unfixed, state (DeVries, 1949, Hilding, 1952, Tonndorf et al, 1962)

Chemical analysis of the fluid on the two sides of the tectorial membrane is most difficult because the attachment to Hensen's cells is very tenuous If this is not done with a great deal of caution it is very easy to end up with endolymph on both sides as Flock (1973) has demonstrated In an extensive study of the tectorial membrane of the rat, Ross (1974) reports that "although the tectorial membrane contains essentially the same ions as endolymph, it does not possess them in similar relative concentrations on both sides of the membrane" (p 460) She states further that "it [tectorial membrane] stands as a barrier between regions of different electrolyte composition to retard diffusion of ions from the one region to the other"

Finally, there is the question of how the tunnel fluids and the subtectorial-inner sulcus fluids communicate Preston & Wright (1974) have presented scanning electronmicrographs showing openings in the head plates of the pillar cells The possibility of fluid transport between the tunnel space and subtectorial space is learly indicated

he tectorial membrane is thus in a position take an active part in the biophysical action the organ of Corti Many possibilities have been outlined (Lawrence, 1967*a*) but it seems apparent that, at the molecular dimensions of fluid movement at hearing threshold, a reaction other than the mechanical movement of stereocilia must exist It may be that there is a variable resistance but the locus of this is more likely the tectorial membrane than the reticular lamina The hairs of the hair cells and the tectorial membrane are bound together and their attachments appear as one cell coat bound to another (Ross, 1974, Borghesan, 1950)

It is certainly conceivable that the first transfer of vibratory energy from the footplate of the stapes is through the tectorial membrane – hair cell complex and that the initiation of any receptor potential is brought about by changes in the permeability of the thin basal body area at the hair-bearing end of the hair cell These changes in permeability are produced by the shuttling of ions within the tectorial membrane enhanced by the positive potential on the endolymphatic surface as the molecular displacement occurs in the highly hydrolysed substance, as Naftalin (1965) has proposed The hair cell itself, thus having its internal homeostasis shifted releases its chemical stimuli to the nerve ending

ZUSAMMENFASSUNG

Unter ständiger Beobachtung mit Hilfe eines „closed-circuit Fernseh Monitors" wurden Elektroden in die Flussigkeitsraume des Cortischen Organes eingefuhrt. Die Ruhepotentiale wurden in Bezug auf die neutrale Spannung des Meerschweinchenkorpers gemessen und mit den anderen Potentialen des Gehorlabyrinths verglichen Man sah, dass die Ruhepotentiale des subtectorialen Raumes des Sulcus internus, des Tunnels, und der anderen Räume des Cortischen Organes gleich oder etwas mehr negativ sind als das Perilymph Potential. Die Deckmembran isoliert die lamina reticularis von der Endolymphe, was beweisst, mit Rücksicht auf andere Zustände dass die Funktion der Deckmembran mehr biophysikalisch als mechanisch ist Ein Film zeigt die Zellen und Räume des Cortischen Organes und die Einfuhrung und Stellung der Elektroden

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DISCUSSION

I Friedmann What are the 'pores'? Can they be identified by any other method? Do you consider the intercellular and subtectorial space to contain perilymph?

K H Vosteen Was tun die Microphonics wenn das Vas spirale blockiert wird? Weshalb sagen Sie, die subtectoriale Flussigkeit sei Perilymphe? Der Subtectorial raum hat Kommunikation mit der Skala media und nicht mit der Skala tympani Vielleicht ist es Endolymphe mit besonderer Zusammensetzung wegen der sekretorischen Aktivitat der Enterdentalzellen der Limbus?

M Lawrence (Reply) to Mr *Friedmann* and Mr *Vosteen* We have tried to localize the whereabouts of the electrode by the electrophoretic injection of fluorescent dyes but the prolonged fixation decalcification and dehydration diffused the edge to such an extent that the procedure was useless In answer to your second question I can only say with certainty that the fluid of the subtectorial space and inner sulcus is not endolymph As Mr Engstrom has stated, we cannot call the fluid either endolymph or perilymph and perhaps this particular fluid differs somewhat from the tunnel fluid We have noticed that the extracellular fluids of the organ of Corti tend to be slightly more negative than perilymph, but not nearly as negative as the intracellular potential

Dr Friedmann Preston and Wright have observed many of these pores by scanning electronmicroscopy but I believe they have not found them yet by transmission electronmicroscopy It may be that they have not run a long enough series of sections

To Mr *Vosteen* We have not yet made enough measurements of a c potential in the tunnel with local blocking of the vas spirale as shown in the motion picture to give a clear answer These experiments are now under way measuring the oxygen tension change while simultaneously determining the magnitude of a c potential

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THE ROLE OF THE BINAURAL TEST IN FILTERED SPEECH AUDIOMETRY

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Abstract The value of the binaural resynthesis test administered according to Matzker's principle can be estimated only when a comparable monaural test can be used as a control The most common positive finding in the filtered speech test is asymmetrical discrimination Both parts of the message delivered to either ear must therefore have the same intelligibility in normal material Binaural intelligibility is highly resistant to degenerative changes in the auditory system, for example Positive findings in the binaural test are encountered frequently in brain stem lesions with vascular or traumatic aetiology

Speech audiometry sensitized by frequency filtration is mainly used for the detection of central hearing disorders As a monaural test, usually with low-pass filtration, it has given satisfactory results in many cases of temporal lobe lesions ich involve a poor discrimination in the con- eral ear (Bocca et al , 1954, Jerger, 1960, o & Antonelli, 1963, Hodgson, 1967, n-Bengtsen, 1973) In the case of brain stem lesions, deterioration in discrimination may occur in either ear or in both ears (Calearo & Antonelli, 1968)

The binaural filtered speech test applied in the form of a resynthesis of two bands, according to Matzker's principle, has given especially satisfactory results in cases of brain stem lesions (Matzker, 1958, Hayashi, 1965, Ohta et al , 1967, Lynn et al , 1972, Smith & Resnick, 1972) However, controversial opinions are reported concerning the value of this test Linden (1964) reported that in brain tumour cases the ability to resynthesize speech was reduced to the same percentage value as the intelligibility of filtered speech presented monaurally, and this binaural test system has been abandoned in some places in consequence of this finding (Korsan Bengtsen, 1973, Liden & Korsan-Bengtsen, 1973) However, the binaural speech resynthesis test has a very interesting theoretical basis and we have thus used this test combined with a monaural filtered speech test as a routine technique for the past 10 years in over 2 000 cases of various peripheral and central lesions in order to ascertain its advantages We shall present now a preliminary report on our findings

METHOD

The filtered speech test used in this study is based on the discrimination of two bands of speech, 480–720 Hz and 1 800–2 400 Hz (Palva, 1965) Each band alone gives the same discrimination of about 15–20 % When the bands are presented together, monaurally, or binaurally, each band to either ear, the level of discrimination rises to about 80 % in normal young people

Word lists are used so that the test words are presented to the subject automatically in the following order (Fig 1) The first word is given to the right ear on both bands, the second to the left ear on both bands, the third word binaurally one band to the right ear and the other to the left, the fourth word in a similar manner to the first and so on With this arrangement the monaural test on both ears and the binaural speech resynthesis test run simultaneously and so are mutually comparable Thus the effect of learning during the test may also be taken into consideration

Each test on the right ear, on the left ear and binaurally, is carried out using 90 words One

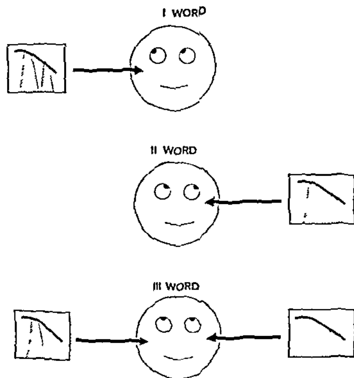


Fig 1 Principal arrangement of the filtered speech test.

sensation level of 50 dB is used Cross-hearing is eliminated by using insert-type earphones No patients with a pure tone threshold poorer than 30 dB were tested.

The test yields three discrimination percentages

$\frac{\text{Right ear}}{\text{Left ear}}$ Binaural

The following may be presented as examples of the principal types of findings

$\frac{80}{75}$	80	Normal discrimination
$\frac{30}{36}$	40	Poor discrimination
$\frac{40}{72}$	44	Asymmetry in monaural test combined with poor binaural discrimination
$\frac{40}{72}$	70	Asymmetry in monaural test combined with good binaural discrimination
$\frac{76}{70}$	36	Binaural test positive
$\frac{36}{42}$	70	Poor monaural discrimination combined with good binaural discrimination

Asymmetry in the monaural test indicates a difference in discrimination exceeding 15%. The binaural test is positive when the binaural discrimination is more than 10% worse than that of the weaker ear in the monaural test If a positive binaural test is combined with asymmetry in the monaural test, its positive nature may be considered doubtful

RESULTS

The test was standardized using 289 healthy subjects Age is seen to have a clear influence on discrimination (Fig 2) The test is also seen to be useful for children

Matzker obtained positive results in cases of presbyacusis Our results (Palva & Jokinen, 1970) show that the most important finding in old people is asymmetry in the monaural test exceeding 15% in half the cases aged over 60

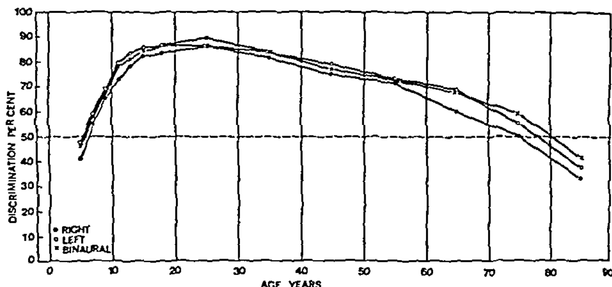


Fig 2 Effect of age on the filtered speech test.

years (Fig 3) Binaural discrimination followed that of the better ear in half of the asymmetrical cases In 20 % the binaural discrimination was over 10 % better than that of the better ear, and only in one case was the binaural discrimination over 10 % worse than that of the poorer ear In this case the asymmetry in monaural imination was 18 %

ipheral diseases such as Menière's disease umatic cochlear hearing loss give positive esults in the monaural test in many cases In these cases binaural discrimination is reduced to the same level than that of the poorer ear In cases of a suspected central lesion, these peripheral diseases must be excluded by means of other audiological and/or vestibular tests

In the cases of central lesions positive findings were most common in the monaural test For cortical lesions these were contralateral and for brain stem lesions, bi-, contra- or homolateral

In 22 cases of intracranial tumours the most common findings were asymmetry in the monaural test (in 6 cases) and bilaterally reduced discrimination (in 4 cases) Only in one case (meningeoma of the olfactory region) was the binaural test positive In 4 asymmetrical cases the binaural discrimination was close to that of the better ear Two of these tumours were infratentorial and two supratentorial

Among 39 cases of multiple sclerosis, 2 patients were found with a positive binaural test and 2 with asymmetry in the monaural test In the latter 2 cases the binaural discrimination was on the same level as the monaural discrimination in the poorer ear In 3 cases the binaural discrimination was over 10 % better than the better monaural score

The filtered speech test was performed on 76 patients with various complaints after a skull trauma (cerebral contusion or concussion, skull base fracture) The binaural test was positive in 7 cases Asymmetry was found in 9 cases, in 4 of which it was combined with poor binaural discrimination (Fig 4) In one case a high binaural score was detected in association with bilateral poor monaural discrimination

The largest number of cases with a positive binaural test were found among patients with various intracranial vascular disorders The binaural test was positive in 26 of the 169 patients in this group (Fig 5) Nine of them had typical symptoms of vertebro-basal insufficiency The most common complaint in patients with a positive binaural test was vertigo Asymmetry was found in 29 cases and poor discrimination *in all*



Fig 3 Asymmetry in the monaural test at various ages Total number of subjects 289

three parts of the test in 11 cases Thus asymmetric finding was connected with poor binaural discrimination in 11 cases and with good binaural discrimination similarly in 11 cases A binaural score more than 10 % higher than the monaural one was found in 3 cases

COMMENT

The most common positive finding in the filtered speech test is asymmetrical discrimination Both parts of the message delivered to either ear must therefore have the same intelligibility

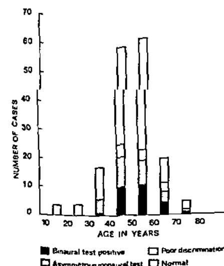


Fig 4 Principal findings in the filtered speech test in 169 ...

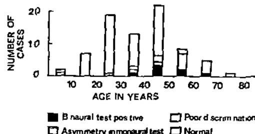


Fig 5 Principal findings in the filtered speech test in 76 patients with late symptoms after skull trauma

in normal material Otherwise no evaluation of the binaural test is possible as unilaterally reduced discrimination may give a faulty positive result

Our findings confirm the opinion that positive binaural results can be obtained, though this situation is rare and is usually connected with brain stem lesion

Monaural deterioration in discrimination may be a consequence of lesion at any level of the perceptive auditory system In these cases it may be useful to observe the binaural discrimination It is logical that in lesions peripheral to the areas responsible for binaural hearing the binaural score should deteriorate to about the same level as the monaural one In higher central lesions the binaural discrimination is often well preserved, even surprisingly high One explanation for this phenomenon is that a monaurally presented signal is handled mainly unilaterally in contralateral auditory areas, whereas a binaurally presented, fused signal spreads more widely in the auditory pathways and may be handled in either or both hemispheres, thus giving the best conditions for discrimination

RÉSUMÉ

On peut estimer la valeur d un épreuve d integration biauriculaire faite selon le principe de Matzker seulement à l aide d un test de controle monauriculaire adéquat Etant donné que le cas anormal trouvé le plus fréquemment dans un examen audiométrique avec la voix filtrée est une discrimination asymétrique de la ...

examen biauriculaire surtout dans les lésions du tronc cerébral causées par des maladies vasculaires et traumatiques.

ZUSAMMENFASSUNG

Der Wert des binauralen Horsynthesetests nach Matzker s Prinzip kann nur durch Vergleich mit einem entsprechenden monauralen Test bewertet werden. Der gewöhnlichste Befund in der Audiometrie mit filtrierter Sprache ist die Asymmetrie Darum müssen die beiden Hälften der Sprache eine gleichwertige Verstandlichkeit in normalen Material haben Das binaurale Horvermögen ist gegen altersbedingte Veränderungen im Horsystem sehr widerstandsfahig. Die meisten positiven Binauraltestausfälle sind bei Patienten mit Hirnstammkrankheiten zu finden

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DISCUSSION

A J Fourcin Were you able to find differences in the type of speech sound receptive errors as a function of site of lesion?

A Palva (Reply) to Mr *Fourcin* No differences in discrimination of various speech sounds as a function of the localization of lesion was detected

Acta Otolaryngol 79 315–318, 1975

ENDOKRINOLOGISCHE HINWEISE FÜR DIFFERENTIALDIAGNOSTIK UND VERLAUFSKONTROLLEN RAUMFORDERNDER INTRASELLÄRER PROZESSE

R Albrecht

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Abstrakt Bei der transsphenoidalen Operation intrasellarer chromophober Hypophysenadenome wurde überraschend 5 mal nur Liquor als Sellainhalt gefunden 3 mal in geschlossenen Zysten 2 mal mit Kommunikation mit dem endocraniellen Liquorraum Nur in einem Fall konnte die Zugehörigkeit zum Krankheitsbild der basalen Arachnitis zystica nachgewiesen werden Endokrinologisch war nur 1 Fall normal Vier zeigten die gleichen hypophysären Hypo- oder Dysfunktionen z.T nur geringen Ausmaßes wie sie bei frühen chromophoben Adenomen gesehen werden, obwohl bei einem Teil makroskopisch kein hypophysäres Gewebe gefunden wurde Die Endokrinologie vermag trotz aller Fortschritte differentialdiagnostische Unterschiede zwischen „leerer Sella" und chromophobem Adenom nicht zu geben

Dagegen erweist sich die wiederholte Somatotropinbestimmung bei der Akromegalie für Frühdiagnose Leistungskontrolle der Operation und in der Nachsorge als sehr wertvoll — (Demonstration einiger Beispiele)

Wenn hier über intrasellare Prozesse gesprochen wird, liegt es in diesem Land besonders nahe, Nager s (1970) großartiger Semon-lecture in London 1939 zu gedenken über den „Paranasalen Zugang zu intrasellaren Geschwülsten" Seine jetzt 35 Jahre alten Ausführungen sind unverändert gültig und geeignet, allen, die neu beginnen, als Grundinformation zu dienen Der Wettstreit zwischen Neurochirurgie und Rhinologie um die operative Therapie und die Bemühungen um eine sinnvolle Abgrenzung sind unverändert im Gange Beide haben ihre Operationstechniken verbessert wobei wir durch die Mikrochirurgie des Ohres sehr viel auch für die Hypophyse gelernt haben

Der Strahlentherapeut ist inzwischen rivalisierend hinzugekommen Aber grundlegende Fortschritte hat nur die Endokrinologie aufzuweisen, die heute ganz anders als vor 35 Jahren hormonelle Veränderungen und Störungen objektivieren kann Es liegt deshalb nahe, ständig zu prüfen, wieweit sie für intraselläre Prozesse aussagekräftig und für die operative Praxis von Wert ist

Es ist dies die Aufgabenstellung dieses Beitrages, allerdings begrenzt auf zwei Fragestellungen
1) Die leere Sella und ihre endokrinen Befunde,
2) Wert hormoneller Verlaufskontrollen beim eosinophilen Hypophysenadenom

Zu 1) Die leere Sella und ihre endokrinen Befunde

Die transsphenoidale Hypophysenchirurgie ist nur sinnvoll, wenn der krankhafte Prozess so frühzeitig erfasst wird, daß er sich im wesentlichen noch auf die Sellaregion beschränkt Aber jede Frühdiagnose büßt, mit wenigen Ausnahmen, gegenüber dem vollentwickelten Krankheitsbild an Sicherheit ein Dies gilt selbstverständlich auch für raumfordernde intraselläre Prozesse, die in der Mehrzahl Ausdruck chromophober Hypophysenadenome sind, besonders wenn typische Symptome der hormonaktiven Adenome fehlen Die große oder deutlich erweiterte Sella wird oft nur zufällig gefunden bei systematischer Suche nach der Ursache von Kopfschmerzen Sofern allgemeiner Hirndruck als Ursache ausscheidet, drängt sich, zumindest bei zunehmender Erweiterung der Sella, der Verdacht eines raumfordernden intrasellaren Prozesses in den Vordergrund Sofern Anzeichen

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A Palva (Reply) to Mr *Fourcin* No differences in discrimination of various speech sounds as a function of the localization of lesion was detected

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ENDOKRINOLOGISCHE HINWEISE FÜR DIFFERENTIALDIAGNOSTIK UND VERLAUFSKONTROLLEN RAUMFORDERNDER INTRASELLÄRER PROZESSE

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Abstrakt Bei der transsphenoidalen Operation intrasellarer chromophober Hypophysenadenome wurde überraschend 5 mal nur Liquor als Sellainhalt gefunden 3 mal in geschlossenen Zysten, 2 mal mit Kommunikation mit dem endocraniellen Liquorraum Nur in einem Fall konnte die Zugehörigkeit zum Krankheitsbild der basalen Arachnitis zystica nachgewiesen werden Endokrinologisch war nur 1 Fall normal Vier zeigten die gleichen hypophysären Hypo- oder Dysfunktionen z T nur geringen Ausmaßes wie sie bei frühen chromophoben Adenomen gesehen werden, obwohl bei einem Teil makroskopisch kein hypophysäres Gewebe gefunden wurde Die Endokrinologie vermag trotz aller Fortschritte differentialdiagnostische Unterschiede zwischen „leerer Sella und chromophobem Adenom nicht zu geben

Dagegen erweist sich die wiederholte Somatotropinbestimmung bei der Akromegalie für Frühdiagnose Leistungskontrolle der Operation und in der Nachsorge als sehr wertvoll — (Demonstration einiger Beispiele)

Wenn hier über intraselläre Prozesse gesprochen wird, liegt es in diesem Land besonders nahe, Nager s (1970) großartiger Semon lecture in London 1939 zu gedenken über den „Paranasalen Zugang zu intrasellären Geschwulsten" Seine jetzt 35 Jahre alten Ausführungen sind unverändert gültig und geeignet, allen die neu beginnen, als Grundinformation zu dienen Der Wettstreit zwischen Neurochirurgie und Rhinologie um die operative Therapie und die Bemühungen um eine sinnvolle Abgrenzung sind unverändert im Gange Beide haben ihre Operationstechniken verbessert, wobei wir durch die Mikrochirurgie des Ohres sehr viel auch für die Hypophyse gelernt haben

Der Strahlentherapeut ist inzwischen rivalisierend hinzugekommen Aber grundlegende Fortschritte hat nur die Endokrinologie aufzuweisen, die heute ganz anders als vor 35 Jahren hormonelle Veränderungen und Störungen objektivieren kann Es liegt deshalb nahe, ständig zu prüfen, wieweit sie für intrasellare Prozesse aussagekräftig und für die operative Praxis von Wert ist

Es ist dies die Aufgabenstellung dieses Beitrages, allerdings begrenzt auf zwei Fragestellungen

1) Die leere Sella und ihre endokrinen Befunde,
2) Wert hormoneller Verlaufskontrollen beim eosinophilen Hypophysenadenom

Zu 1) Die leere Sella und ihre endokrinen Befunde

Die transsphenoidale Hypophysenchirurgie ist nur sinnvoll, wenn der krankhafte Prozess so frühzeitig erfasst wird, daß er sich im wesentlichen noch auf die Sellaregion beschränkt Aber jede Frühdiagnose büßt, mit wenigen Ausnahmen, gegenüber dem vollentwickelten Krankheitsbild an Sicherheit ein Dies gilt selbstverständlich auch für raumfordernde intraselläre Prozesse, die in der Mehrzahl Ausdruck chromophober Hypophysenadenome sind, besonders wenn typische Symptome der hormonaktiven Adenome fehlen Die große oder deutlich erweiterte Sella wird oft nur zufällig gefunden bei systematischer Suche nach der Ursache von Kopfschmerzen Sofern allgemeiner Hirndruck als Ursache ausscheidet, drängt sich zumindest bei zunehmender Erweiterung der Sella der Verdacht eines raumfordernden intrasellären Prozesses in den Vordergrund Sofern An[illegible]chen

für eine Hypo- oder Dysfunktion der Tropinfunktionen der Adenohypophyse nachweisbar werden, zögern wir nicht, transsphenoidal die Sella zu revidieren, selbst wenn ophthalmologische und neurologische Hinweise fehlen In der Regel stoßen wir auf chromophobe Adenome, aber gerade in letzter Zeit haben wir 5 mal gänzlich unerwartet, trotz deutlich veränderter Sella, diese ohne jegliches Gewebe, aber voller farbloser wässriger Flüssigkeit gefunden, die wir *nach unseren Analysen als Liquor* bezeichnen mussen Hierbei ist hervorzuheben, daß 3 mal ein abgeschotteter Liquorraum vorgelegen haben muß, da sich nur eine begrenzte Menge Flussigkeit abpunktieren ließ, die Hypophysendura danach faltig zusammenfiel und die Sella sich nicht nachfüllte Hier haben wir den Sack eroffnet und die Innenseite inspiziert Von Hypophysengewebe wurde keine Andeutung gefunden Die Proben aus der Wandung entsprachen histologisch kleinen Durastücken Einmal wurde histologisch von bindegewebiger Wand einer Zyste gesprochen

Ophthalmologisch fand sich einmal eine flüchtige Sehfeldeinschrankung bds, Zisterno- und Arteriogramme waren in jedem Fall unauffällig Endokrinologisch bot eine Patientin keine eränderungen In beiden anderen Fällen be- nd eine sichere hypophysäre Hypofunktion

Im 4 und 5 Fall füllte sich die Sella trotz Entleerung von 15 ccm Liquor immer wieder nach, so daß wir eine Kommunikation mit dem freien Liquorraum annehmen müssen Hier haben wir die Dura nicht weiter eröffnet und können deshalb nichts uber Fehlen oder Anwesenheit von Hypophysenresten in der Sella aussagen Endokrinologisch bestand eine deutliche hypophysäre Hypofunktion und einmal schwere zunehmende Visusbedrohung, im anderen nur temporärere Gesichtsfeldeinschränkung Eine Erklärung für die Entwicklung der „leeren Sella" haben wir nur in dem einen Fall, der durch schnelle Visusbedrohung auffiel Hier wurde neurochirurgisch interveniert und das Bild der basalen Arachnitis zystika gefunden, mit kastaniengroßer Liquorzyste, teils intra- teils suprasellärer Ausdehnung Die restlichen 4 Patienten unterscheiden sich in Anamnese, Symptomatologie, klinischen und röntgenologischen Befunden nicht eindeutig von chromophoben Adenomen Inbesondere endokrinologische Analysen machten eine praeoperative Unterscheidung nicht möglich Roth et al (1971) haben in 3 Fällen, Caplan & Dobben (1969) in 5 Fällen über die Pneumencephalographie die „leere Sella" diagnostizieren können Friedmann & Marguth (1961) haben bereits 1961 über 8 Pa*tienten mit intrasellärer Liquorzyste berichtet*, bei denen die endocranielle Operation fast regelmäßig Arachnoidalzysten aufdeckte Sie heben hervor, daß weder klinisch-endokrinologisch noch vom Röntgenbefund her, eine sichere Möglichkeit besteht, derartige Zysten von soliden Hypophysentumoren zu unterscheiden Wir mussen diese Feststellung voll bestätigen Endokrinologisch sind wir zwar immer wieder überrascht, wieweit funktionsfähiges Hypophysengewebe reduziert sein kann, bei nur relativ geringfugiger hormonaler Hypo- oder Dysfunktion Offensichtlich ist diese nur eine unspezifische Antwort, bei der es gleichgultig ist, ob die Funktionsminderung durch einen chromophoben Tumor, durch hypophysäre Zysten oder durch äußere Druckatrophie bedingt wird

Da aber die Berichte über die „leere Sella" in letzter Zeit häufiger zu werden scheinen (Bernasconi et al, 1972, Busch, 1951, Hodgson et al, 1972, Lee & Adams, 1968) verdient sie unsere Aufmerksamkeit Möglicherweise ist über eine verfeinerte Röntgendiagnostik mehr Aussage zu erwarten als über weitere hormonale Analysen, es sei denn, daß wir eines Tages spezifische Auswirkungen des chromophoben Adenoms erkennen lernen

Zu 2) Wert hormonaler Verlaufskontrollen beim eosinophilen Hypophysenadenom

Wahrend uns die Endokrinologie zur präoperativen Differenzierung zwischen frühen intrasellaren chromophoben Adenomen, hypophysären Zysten und „leerer Sella" z. Zt noch enttäuscht, ist sie uns bei der Akromegalie gezielt behilflich

Es ist bekannt, daß die somatischen und viel faltigen hormonellen Veränderungen der Akromegalie nach Hypophysektomie selten voll rückbildungsfähig sind. Deshalb streben wir auch für sie z. Zt. eine frühe Operation an, obwohl das eosinophile Adenom keine starke örtliche Destruktionstendenz zeigt. Aber Andeutungen einer Akromegalie sind klinisch nicht immer eindeutig und so festumrissen, wie dieses Krankheitsbild allgemein gesehen wird, ist es viel doch nicht. So sind akromegale Begleiterscheinungen bei „leerer Sella" gesehen worden (Friedmann & Marguth, 1961). Es sei auch auf die eigenartigen gemischzelligen Adenome hingewiesen, deren endgültige Zuordnung heute von electronenmikroskopischen Studien erhofft wird (Kinnman, 1972). So ist die Möglichkeit der Somatropinbestimmung und die Prüfung eines Spiegels unter Hypoglykämie für diese ruhen und atypischen Fälle eine willkommene und wertvolle Hilfe. Die praeoperative STH Bestimmung erlaubt uns auch, die Auswirkung der Operation zu kontrollieren, zumal heute die Tendenz besteht, nur das Adenom zu entfernen und zumindest die Neurohypophyse zu erhalten. Sie hilft im Laufe der postoperativen Nachsorge uns immer wieder, vom Dauererfolg oder einem aufkommenden Rezidiv zu unterrichten, wie 3 typische Beispiele zeigen mögen.

Typisches Beispiel

E 47 Jahr ♂. Typisches histologisch bestätigtes eosinophiles Adenom, bei dem versucht wurde die Neurohypophyse zu erhalten mit mehrfachen endokrinen Störungen mittelgroßer Struma pathologischem Probetest, erheblicher Hyperchlorhydrie und stark erhöhten Somatropinwerten. Postoperativ Fortbestehen einer latenten Diabetesdisposition. Im Metoprontest noch nachweisbare ACTH Produktion, STH Werte dagegen an der unteren Grenze der Norm, die sich im Laufe der Nachkontrollen bisher nicht änderten.

Typisches Beispiel

H 42 Jahr ♂. Klinisch stark ausgeprägte Akromegalie, Diabetes mellitus, Narkolepsie und psychische Veränderungen bei nur leicht ballonierter Sella. Endokrinologisch Alkalische und anorganische Phosphatasen im Normbereich. Im Metopirontest gute ACTH Ausscheidung. Grundumsatz noch normal. STH Werte dagegen stark erhöht und keine Suppression unter Hypoglykämie. Die Operation trifft typisches Tumor... histologisch aber als chromophobes ..., das nur in den Randgebieten Zellen zeigt. Postoperativ Gesamt... gebessert, selbst narkoleptische An... die aber nach einem Jahr wieder ... starker nächtlicher Hyperhidrosis. ... sich im Bereich der Norm. Patient als rezidivgefährdet angesehen ... typischen Symptomatik und der auffäl...

... piel

... seit Jahren akromegale Veränderungen ... Stoffwechselkrisen, besonders diabetische. ... wird ein kleinzystisch gekammerter Hypophysentumor gefunden, der histologisch schwer zu ..., aber doch als sekundär verändertes eosinophiles Adenom angesehen wird. Postoperativ immer wieder ... schwere diabetische Krisen, die schwer beeinflußbar sind. Bereits 1 J. p. op. wieder hohe STH Werte. Deshalb nochmals transsphenoidale Revision der Sella, die aber sekundär so stark verschwartet ist, daß abgesehen von ...

... labil und 3 Jahre nach 1. Op. kommt Patientin durch einen Herzinfarkt bei stenosierender Coronarsklerose und Cor bovinum ad exitum. In sectione kirschgroßes Rezidiv eines eosinophilen Adenoms.

Für die Nachsorge chromophober Adenome fehlen uns noch schmalbandige, spezifische Teste, so daß wir genötigt sind, in Intervallen komplex röntgenologisch ophthalmologisch, neurologisch und endokrinologisch nachzuuntersuchen, um etwaige Rezidiv früh zu erfassen oder uns von der Dauerheilung zu überzeugen.

SUMMARY

In expectance of early chromophobe adenomas ... transsphenoidal intervention revealed ... spinal fluid only 3 times in closed ... connection to the endocranial space ... neuro-surgical approach revealed ... Endocrine studies detected no ... tween empty sella patients and ... mata. Compared with this in ... measurement of the different ... gland and especially the repeated ... proved most effective for ... operative effect and long-term ...

RÉSUMÉ

En expectant des adenomes ... sphenoidal a decele et ...

phalorachid en dans la loge hypophysaire élargie trois fois dans des kystes isolées, deux fois avec communication endocranielle Dans un de ces cas l intervention neurochirurgical a revélé une arachnitis kystique basale Les symtômes de ces malades ne differaient pas comparé avec des malades des adenomes chromophobes ou des kystes hypophysaires Meme les alterations endocrinien nes étaient semblable et une exacte differenciation pre opératoire n etait pas possible

Contre cela la détermination repéte des hormones pituitaire particulier du hormone somatropique se mon trait tres utile pour la diagnose en cas atypique, pour examen de l effectivite operatoire et pour controler guérison délinie ou pour trouver une récidive precoce

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ELECTRONYSTAGMOGRAPHY IN THE PATHOLOGY OF THE CENTRAL NERVOUS SYSTEM

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Abstract The program of the electronystagmographic examinations in the pathology of the central nervous system contains 11 groups of tasks Sixty signs of central impairments are listed, from which 26 nystagmic irregularities had a small diagnostic value No sign occuring alone could be taken as pathognomonic, only a group of signs can be used for the impairment localization Besides for the peripheral vestibular and the peripheral vestibulocochlear impairments, the authors succeded to make a group containing pathognomonic signs for mixed vestibular impairments as well as for multiple sclerosis affecting vestibular structures

Perhaps the main problem and task of electronystagmography today is the diagnosis of impairments in the CNS After 10 years of work and recording over 6 000 electronystagmograms we decided to subject our data to a systematic analysis and to compare them with proved diagnoses, with the findings of neuro surgeons

Since there is considerable controversy concerning the findings and experience of various authors, they cannot be considered a reliable basis on which to make analyses Our attitude to our own findings and results is equally critical There is justification for a comparison between a bundle of nerve fibres, ganglion cells and synapses with neurotransmitters on the one hand and a complex electronic instrument on the other Still, it would be naive to believe that a disorder can be easily detected with the methods used by an electronics expert Yet we are far from claiming that all the possibilities that the electronystagmographic record can offer have been exploited It does contain and display signs which, as yet, we have not learned how to read

On the basis of our experience and the experience gained by others we have prepared a list of signs indicative of peripheral and central impairments The signs of peripheral impairments will not be mentioned here

The signs which indicate central impairment are

1 Spontaneous nystagmus in the direction of the weaker labyrinth, and not in the direction of the healthy one
2 Spontaneous nystagmus which does not become weaker on fixation
3 Spontaneous nystagmus which increases on fixation
4 Vertical nystagmus (multiple sclerosis)
5 Retraction nystagmus
6 Oblique nystagmus
7 Rotatory nystagmus (medulla oblongata)
8 Square waves (atherosclerosis of the brain)
9 Restlessness of the eyes
10 Vertical gaze nystagmus supranystagmus in supraversion
11 Bitemporal gaze nystagmus dextronystagmus in dextroversions and levonystagmus in levoversions
12 Centrifugal nystagmus dextronystagmus in dextroversions, levonystagmus in levoversions, supranystagmus in supraversions, or in all directions (mesencephalic and pontine impairments, inferior cerebellar fossa)
13 Positional nystagmus type Nylen I persistent, unfixed, indefatigable nystagmus (inferior cerebellar fossa)
14 Positional nystagmus type Nylen II persistent, fixed, indefatigable nystagmus

phalorachidien dans la loge hypophysaire élargie, trois fois dans des kystes isolées, deux fois avec communication endocranielle Dans un de ces cas l intervention neurochirurgical a revélé une arachnitis kystique basale Les symtômes de ces malades ne differaient pas comparé avec des malades des adénomes chromophobes ou des kystes hypophysaires Meme les altérations endocriniennes étaient semblable et une exacte differenciation pré operatoire n'était pas possible

Contre cela la determination répété des hormones pituitaire, particulier du hormone somatropique se montrait tres utile pour la diagnose en cas atypique, pour examen de l effectivité operatoire et pour contrôler guérison definie ou pour trouver une récidive précoce

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8 FITZGERALD AND HALLPIKE'S CALORIC ENG TEST

ny			d	a	f	s	v	II
	1 test	NF						
		F						
	2 test	NF						
		F						
	3 test	NF						
		F						
	4 test	NF						
		F						
	5 test	NF						
		F						
	6 test	NF						
		F						

7 directional preponderance s R L %
d R L %

8 diminished excitability s R L %
d R L %

Fig 1g

9 OPTOKINETIC ENG TEST

ny		a	f	s
	1 nystagmus to the R			
	2 nystagmus to the L			

3 weaker nystagmus to the R L %
4 optokinetic inversion R L

10 ROTATIONAL ENG TEST

ny			d	s
	1 rotation to the right side	Dny		
		Lny		
	2 rotation to the left side	Lny		
		Dny		

Fig 1h

11 ENG FINDING

1 normal ENG
2 peripheral impairment D L
3 central impairment D L
4 non-specific ENG

Fig 1i

29 Fixational suppression of nystagmus absent
30 Negative fixational suppression of nystagmus stronger nystagmus on fixation than on non fixation
31 Change in the direction of nystagmus during fixation
32 Nystagmic irregularities only in one direction of the induced nystagmus
33 Pathologic fixational suppression in only one direction of the induced nystagmus
34 Change in the direction of nystagmus during fixation in one direction of the nystagmus only
35–60 Nystagmic irregularities

IN

at Dny — at Lny

1 Rounded transitions between the quick and slow component

Rounded transitions between the slow and quick component

3 Absence of the slow component /asymmetria/

4 Absence of the quick component /asymmetria/

5 Dysrhythmia a s /f relatively constant/

Fig 2a

IN

at Dny — at Lny

6 Dysrhythmia a f /s relatively constant/

Pause

8 Counter jerk

9 Notch in the slow component /Abortive quick component/

10 Notch in the quick component /Abortive slow component/

Fig 2b

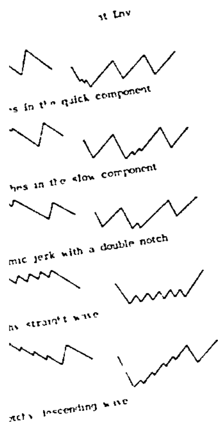


Fig 2d

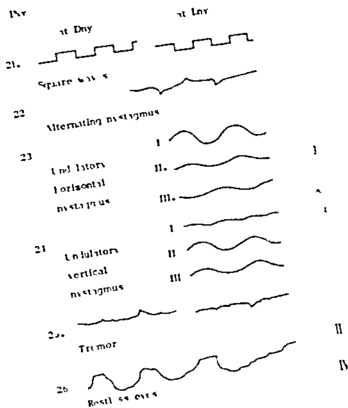


Fig 2e

Fig 1 shows the routine examinations performed in all our patients The findings in each patient were marked by encircling the numbers The data on the acuteness of hearing, on the ENG fixation tests, positional tests, caloric and rotation tests are entered separately for the direction, amplitude, frequency and speed of the slow component, and the figures on the left side show nystagmic irregularities (iny) in three degrees

As shown in Fig 2, we have listed 26 different (iny) nystagmic irregularities

In our study the findings of 50 neurosurgical patients were analysed The neurosurgeon prepared a short summary of the neurosurgical findings and the discharge diagnosis for each patient According to need we checked the accuracy of single signs also in some of our other patients for whom we had reliable neurosurgical and neurological diagnoses The data presented in Figs 1 and 2 are displayed on the ordinate and the number and diagnosis of patients on the abscissa

PERIPHERAL VESTIBULAR IMPAIRMENT
/Neuronitis vestibularis/

1 VERTIGO towards the diseased side
NAUSEA vomiting
3 NYSTAGMUS towards the healthy side
4 ATAXIA towards the d seased s de
5 VESTIBULAR EXCITABILITY AB ENT or d m nished on the caloric eng test
6 Hearing normal

Fig 3

PERIPHERAL VESTIBULOCOCHLEAR IMPAIRMENT
/Meniere s disease vascular inflammatory or traumatic impairment of the labyrinth/

1 VERTIGO towards the diseased side
NAUSEA vomiting
3 NYSTAGMUS towards the healthy side
4 ATAXIA towards the d seased s de
VESTIBULAR EXCITABILITY AB ENT or n nished on the caloric test
HARD HEARING OR DEAFNE , s n ors

Fig 4

On the basis of our analyses the patients were divided into four groups

I Patients with no CNS impairment
II Patients with mixed (peripheral and central) vestibular impairments tumours of the vestibulocochlear nerve and pontocerebellar tumours
III Patients with multiple sclerosis affecting vestibular structures
IV Patients with other central impairments

Due to the lack of time, instead of discussing single findings or groups of data the figures containing pathognomonic signs will be presented (Figs 3–6)

Figs 3 and 4 will be left without comment as they do not directly concern the subject of our consideration

Those signs which were always present in the tumour of the vestibulocochlear nerve or in the pontocerebellar tumour (Fig 5), and as a group do not occur in any other case, were capitalized in the figure

The caloric test cannot be replaced by the rotation test, and the rotation or pendular test may reveal compensation but cannot yield the information necessary for a reliable diagnosis

In multiple sclerosis (Fig 6), fixational suppression is, as a rule, negative, and only rarely absent, it has undergone a more severe pathologic alteration than in the previous group In this group centrifugal nystagmus is also more marked in at least three directions

We had expected that with a similar probability for correct diagnosis we can also prepare tables for the infratentorial impairments and a separate table for supratentorial impairments However, this time our attempt failed

MIXED VESTIBULAR IMPAIRMENT
/Tumor of the ve t bulor ch l r n rv or pontoc rebell r tum r/

1 CENTRIFUGAL NYSTAGMU b t npor l r r t
2 CORNEAL REFLEX ABSENT or we k
3 VESTIBULAR NON EXCITABILITY r t t nt ll l m n t exc t bil ty on the caloric te t
4 DEAFNESS OR EVERELY IMPAIRED HEARIN
P tholog c Fixational pr ior li tl x t v b nt or r g t e
6 At x with out l ter l on t o l ff ct or le lth side
pos t m n t g n
OPK n us
rot tor t n us
irre es f n

Fig 5

MULTIPLE SCLE
/af fecti g ve t b l r /

1 CENTRIFUGAL NY
PATHOLOGIC FI
n ative or abse
3 At xia with t
articular l
4 Vestibular e
H aring n

Fig 6

Central infratentorial impairments may, though not always, be manifested by

central nystagmus
weakened corneal reflex
deafness or hard hearing
pathological fixational suppression

Non pathognomic are ataxia, positional nystagmus, optokinetic nystagmus, caloric and rotation nystagmus

Similar data were obtained for supratentorial impairments We were surprised by the lack of sure signs indicative of infratentorial impairments This is an additional argument which necessitates the adoption of the concept of mixed vestibular impairments, characterized by signs of both peripheral and central impairments

We hope that by further analyses, similar to those we have presented but based on a large number of examinees with proved neurological diagnoses, we will be able to prepare a few more diagnostic tables For a greater number of neurosurgical diagnoses, a number of samples several times greater would be necessary Therefore, we restricted our work to groups for which we have reliable data and which were also checked on our other patients

y this analysis we have, perhaps, temporarily ricted our electronystagmographic diagnosis though we believe that we have also made it more reliable Only a reliable basis can assure a faster, safer and greater advancement

ZUSAMMENFASSUNG

Das Programm der elektronystagmographischen Bearbeitung der Pathologie des Zentranervensystem umfasst den Blickrichtungsnystagmus, den Balancetest, das Audiogramm, den ENG Fixationstest den ENG-Lagetest den Hallpikeischen Lagetest den Nackentorsionstest die Fitzgerald und Hallpikeische Thermische Prufung, den Optokinetischen ENG Test so wie die Drehprufung, was zusammenfassend insgesammt 11 Prufungsverfahren ergiebt

Aufgezählt wurden insgesammt 60 Zeichen Zentraler Schädigung, von denen die 26 Unregelmässigkeiten des Nystagmus die in unseren Material vorkommen keinen grossen diagnostischen Wert hatten Kein fur sich allein stehendes Zeichen konnte man zur Diagnostik gebrauchen sondern nur eine Zeichengruppe als solche kann man zur Lokalisierung der Schädigung verwenden

Ausser für die peripheren Vestibularis Störungen und die peripheren Vestibulocochlearen Störungen gelang es derzeit eine bestimmte Zeichengruppe für die kombinierten peripheren zentralen Störungen und fur die Sclerosis Multiplex (wenn sie die vestibulären Strukturen erfasst hat) zu gruppieren

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THE ACTIVITY OF THE STAPEDIUS MUSCLE IN MAN DURING VOCALIZATION

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Abstract The EMG of the stapedius muscle and visible movements in the stapedius tendon during the subject's own vocalization of an [a] and during contralateral acoustic stimulation were studied in subjects with ear drum perforation The threshold for stapedius activity was near the lowest vocal intensity that the subjects could produce At normal vocal effort the stapedius muscle was activated to about 50% of its maximum value The EMG often started before the vocal sound, indicating that the stapedius muscle can be activated from the central nervous system as a part of the vocalization process It is suggested that the contraction of the stapedius muscle during vocalization reduces the masking caused by the low frequency components of the person s own voice even at normal vocal effort and thereby improves intelligibility of simultaneous external speech

The activity of the stapedius muscle in response to sound has been analysed in great detail (for review see Møller, 1972) Several studies have shown that this muscle can also be activated by various non acoustic stimuli In man, cutaneous stimulation around the ear lobe, and movements such as swallowing, yawning, lifting the upper eyelids and forced closure of the eyes have been found to elicit change in the ear s acoustic impedance or to cause elctromyographic activity (EMG) in the stapedius muscle (Metz, 1951, Klockhoff & Anderson, 1959, Klockhoff, 1961, Salomon & Starr, 1963, Djupesland, 1967) It has been observed that the stapedius muscle is active during vocalization both in animals (Carmel & Starr, 1963, Simmons, 1964, Henson, 1965) and humans (Klockhoff, 1961, Salomon & Starr, 1963, Shearer & Simmons, 1965, Djupesland, 1967) Some of these observations are based on recordings of acoustic impedance change of the ear Such measurements are, however, difficult to interpret in detail since the vocalized sound itself directly interferes with the reflex recording More reliable information on the activity in the stapedius muscle during vocalization may be gained from electromyographic recordings EMG studies, which are cumbersome due to the anatomical localization of the stapedius muscle in the ear have in man been made with unipolar electrodes in the stapedius muscle or its tendon and with the reference electrode in the ear lobe Unipolar recordings of this type are likely to be influenced by electric activity generated in neighboring muscles, e g jaw and face muscles It is doubtful that sufficient precautions against such influence have been taken in earlier studies Furthermore the temporal relation of the stapedius activity to the person's own voice and the magnitude of the stapedius response in relation to the vocal intensity are insufficiently known

The aims of the present work were to study

(1) The quantitative relation between the degree of the stapedius activity and the sound level

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of the person's own voice especially with regard to normal vocal effort

(2) The mechanism for the activation of the stapedius muscle during vocalization, particularly its onset

MATERIAL

The experiments were performed in 4 females and 4 males in the age range 14 to 60 years (mean 40 years) Six of the subjects had a large, dry eardrum perforation with visible stapes and stapedius tendon in one ear In this ear the hearing thresholds were elevated to 15 dB HL or more (ISO Standard 1964) in the frequency range 0 125–2 0 kHz The remaining 2 subjects had a transparent eardrum scar adherent to the stapes and stapedius tendon In the other ear, 7 of the subjects had hearing thresholds within 20 dB HL in the frequency range 0 125–2 0 kHz and a normal eardrum or an eardrum scar upon microscopic inspection One subject had bilateral, dry eardrum perforations and air-conduction thresholds elevated to 35 dB HL or more The bone-conduction hearing thresholds were within normal limits to at least 3 0 kHz in both ears for all subjects In 5 of the subjects i eardrum perforation EMG from the dius muscle was obtained during vocaliza- and during contralateral acoustic stimula- n Results from 4 of these 5 subjects during acoustic stimulation have been presented earlier (Zakrisson et al, 1974) In 7 of the 8 subjects the weakest vocal intensity giving visible stapedius muscle contraction was determined

METHODS

Vocalization

The subjects were trained to vocalize an [a] of 1–3 sec duration with a distinct onset and with various vocal efforts The [a] was chosen since it could be produced with an abrupt onset a fairly stable intensity and with minimal activation of jaw-, face- and tongue muscles The subjects kept their mouths slightly open, with the jaw-muscles and face in a relaxed state a few seconds before and during the vocalizatio This posture was important since it was show that electrical activity of the jaw muscles (Fig 1) or face-muscles (Fig 2) might be picked u by the recording electrodes Single words c phrases were also used but the results from thes tests were excluded from the present report, be cause of the difficulty in obtaining rapid onse and stable amplitudes which made the inte pretation of the EMG activity difficult

In 6 of the 8 subjects the lowest and highe vocal intensity producible was also measured i an anechoic chamber for comparison

The vocal sounds were recorded with a Brü & Kjaer condenser microphone (type 413 placed 20 cm in front of the mouth The inten sity was measured by a noise level meter (Brüe & Kjaer, type 2606, fast setting) and recorde on a Brüel & Kjaer level recorder The intensit of each vocalized sound was defined as th smoothed (by eye) maximal value of the re cording from the noise level meter During measurement of stapedius EMG, the speech signal was recorded on one channel of a two-channel tape recorder (Revox, type A 77)

The EMG recordings and the determination of the visible threshold for stapedius muscle contraction were performed in a laboratory with a background noise level below 25 dB (A)

EMG recordings

During vocalization and acoustic stimulation EMG recordings from the stapedius muscle were made in 5 of the subjects with eardrum perforation The method for recording EMG from the stapedius muscle has been described in detail (Zakrisson et al, 1974) A unipolar electrode (Vallbo & Hagbarth, 19(5) was in troduced through the eardrum perforation and inserted into the stapedius muscle near its tendon Attempts to insert two needle electrodes in the stapedius muscle in order to obtain bipolar recordings were not successful Therefore a ref erence electrode had to be inserted in the ipsilateral ear lobe Neither local anesthesia nor sedation of the subject was utilized The EMG signal was recorded on the second channel of

Table I *Stapedius muscle activity determined with EMG and visual observation together with ranges of vocal intensity Eight subjects*

Subj	Vocaliz. (dB SPL) EMG thresh	Vocaliz. (dB SPL) Vis thresh	Vocaliz. level (dB SPL) Min.	Vocaliz. level (dB SPL) Max.	Acoust stim. 0.5 kHz (dB SPL) EMG thresh	Acoust stim. 0.5 kHz (dB SPL) Vis thresh	Max acoust EMG in per cent of max vocaliz. EMG	Acoust thresh minus vocaliz. thresh (dB)
GO	75	82	70	97	111	115	138	36
BS	60[a]	72	62	89	113[d]	115[d]	55	53
VB	60[b]	66	60	95	109		153	49
AH	74	76	69	101	92	97	100	18
ES	(83)[c]		79	97	96		82	
AN		76	69			85		9
MG		65	64			130		65
GS		73	73			95		22
Mean	67	73	68	96			106	36

[a] Extrapolated from the 25 % level
[b] Extrapolated from the 47 % level
[c] Corresponds to 48 % of max value during vocalization
[d] 2.0 kHz.

Vocalization levels were measured 20 cm in front of the mouth during the experimental session in the laboratory

the tape recorder The frequency response of the amplifier was 60 Hz – 6 kHz

The integration of the tape-recorded EMG was accomplished by low pass filtering the full-wave rectified EMG signal A low-pass filter with attenuation rate of 18 dB per octave and cut-off frequency of 3 9 Hz (3 dB) was used The amplitudes of the integrated EMG were measured from the level of the electrical background noise to maximum as averaged (by eye) during approximately 0 2 sec (e g , white, horizontal bars of Fig 3) Each value was expressed in per cent of the maximum amplitude obtained during vocalization and plotted as a function of vocal intensity (dB SPL) to yield stimulus–response curves Since the EMG during contralateral stimulation with pure tone at 0 5 or 2 0 kHz was obtained in the same experimental session it was possible to compare amplitudes of the EMG during vocalization and acoustic stimulation

The threshold intensity for stapedius movement

In 7 subjects the threshold for stapedius movement was determined both during vocalization and contralateral acoustic stimulation with the aid of a Zeiss operating microscope at a magnification of 10 or 16 times The lowest vocal intensity or sound stimulus intensity at which movements of the stapes or the stapedius tendon were observed was determined In those subjects where EMG recordings were obtained,

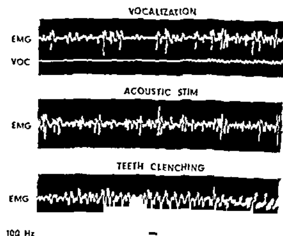


Fig 1 EMG recordings obtained [illegible] electrode in the stapedius muscle and [illegible] reference electrode in the ipsilateral ear lobe [illegible] during vocalization of [a] Record[illegible] the in-tensity of which (97 dB SPL) [illegible] cm in [illegible] of the mouth is shown [illegible] during contralateral acoustic [illegible] (0.5 [illegible] tone at 120 dB SPL) [illegible] ing The same ampli[illegible] EMG [illegible] time scale (indicated [illegible] three graphs Subject [illegible]



Fig 2 EMG recordings obtained with a unipolar electrode in the stapedius muscle and with a reference electrode in the ipsilateral ear lobe Left graph during vocalization of [a] the intensity (86 dB SPL) measured 20 cm in front of the mouth Middle graph during contralateral acoustic stimulation (2 0 kHz pure tone

the threshold values were compared It was found that the visual threshold was higher than the EMG threshold (Table I) This difference was in most cases less than 10 dB This confirms earlier observations by Fisch & v Schulthess (1963)

Control measurements

Since the recordings were made by a unipolar electrode special precautions had to be taken in interpreting the results Therefore recordings were made with one electrode in the stapedius muscle and the other in the ear lobe during strong teeth clenching or facial movements in all subjects These recordings were then compared with the EMG during vocalization and contralateral acoustic stimulation obtained at the same experimental session Figs 1 and 2 show such comparisons from 2 subjects The analyses were made on fast time bases in order to clearly illustrate the shape of the motor unit potentials

It is seen that action potentials appearing during vocalization and acoustic stimulation are distinct repetitively firing have large amplitudes in relation to background noise short durations and contain very fast components whereas during teeth clenching and facial movements there was a continuous irregular activity without distinct action potentials and fast transients Heard through a loudspeaker the difference between these two signals was striking It thus seems justified to conclude that the activity during vocalization and acoustic stimulation originates from the stapedius muscle whereas the activity recorded during teeth clenching and facial movements may be remote activity from the jaw and facemuscles picked up by the two recording electrodes

Great care was taken in the present study to minimize movements of the neighboring muscle groups during vocalization It is therefore concluded that the EMG recordings can form the basis for analysis of the activity of the stapedius muscle during the subject s own vocalization

RESULTS

General characteristics

Fig 3 shows EMG activity (upper traces) and integrated EMG (middle traces) from the stapedius muscle recorded during vocalization of [a] at three different vocal intensities in one subject, as indicated above each graph Recordings of the vocal sound are shown in lower traces The amplitude of each integrated EMG is measured from the horizontal bar to the baseline It is seen that the EMG activity increases as the voice is raised The EMG occurs largely simultaneously with the speech signal yet there is no close correlation between the variation of the integrated EMG amplitude and the variation of the vocal intensity as a function of time Especially at the highest intensities the integrated EMG fluctuates partly independently of the fluctuations in sound level Motor unit potentials are seen after the termination of the speech signal Such afterdischarge in the EMG recordings was seen in all subjects At the highest vocal intensity shown in this figure the afterdischarge lasts for about 300 msec

Spontaneous activity in the stapedius muscle was rarely seen

Relations between stapedius activity and vocal intensity

The amplitude of the integrated EMG (measured as illustrated in Fig 3) is shown in Fig 4

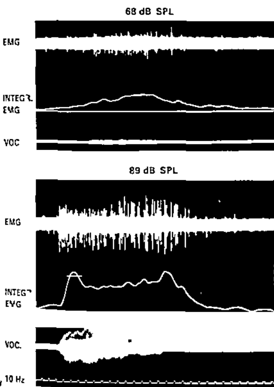


Fig 3 EMG (upper trace) integrated EMG (middle trace) and speech signal (lower trace) at three vocal intensity levels of [a] measured 20 cm in front of the mouth The baseline represents the background electrical noise level Horizontal bars depict the amplitudes used to measure the integrated EMG amplitude Subject B S

as a function of vocal intensity in the 2 subjects GO (left graph) and BS (right graph) when they vocalized [a] with various vocal efforts from the weakest possible to the strongest Each point represents the individual integrated EMG amplitude as a percentage of the maximum value obtained during vocalization EMG amplitudes, calculated as the average value of the individual points within each 4-dB interval, are connected by continuous lines Arrows show the weakest vocal intensity giving visible movement of the stapedius tendon, as observed through the operating microscope

Fig 4 reveals that the EMG activity in general increases as the vocal intensity is increased There is, however, a considerable spread of the data which is most likely due both to an inherent variability of the stapedius activity during vocalization, as well as to uncertainty in the determinations of the integrated EMG amplitudes and the vocal intensity levels, consequent upon their rapidly varying time course The threshold value (defined as the intensity that gave 10 % of maximum EMG amplitude during vocalization) was 75 dB SPL (left graph) and about 60 dB SPL (right graph, extrapolated) The threshold values were thus close to the weakest vocal intensity the subjects could produce in the laboratory (Table I) When the vocal efforts were measured in the anechoic chamber the weakest vocal intensity was somewhat lower, 14 dB (subject G O) and 7 dB (subject B S)

The threshold values for activation of the stapedius muscle as determined with EMG and visual observations are summarized in Table I Thresholds obtained both during vocalization and contralateral sound stimulation and the relations between the corresponding maximum response amplitudes, are included together with the range of vocalized sound intensity (in the experimental situation) Two of the EMG thresholds during vocalization are obtained by extrapolation to 10 % from the 25 % (subject B S) and 47 % (subject V B) level One value (subject E S) corresponds to 48 % of maximum during vocalization In these 3 subjects the EMG threshold could not be established because their vocal intensity during the experiment was not

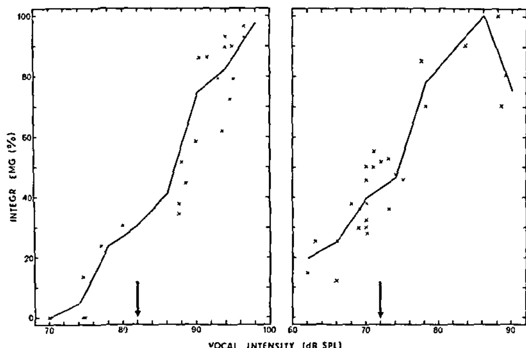


Fig 4 Amplitude of integrated EMG as a function of vocal intensity measured 20 cm in front of the mouth in subject G O (left graph) and B S (right graph) Crosses represent individual values expressed as a percentage of the maximum amplitude obtained during vocalization The continuous lines connect mean valu in 4-dB intervals Arrows indicate the lowest vocal in tensity giving a microscopical visible movement of th stapedius tendon

sufficiently low The acoustic stimulation was 0 5 kHz pure tone in all cases except one (subject B S) from whom no response could be obtained at this frequency due to the pronounced ... ve hearing impairment In this case 2 0 kHz was used

It is also seen in Table I that the threshold for stapedius EMG activity during vocalization is close to the lowest vocal intensity obtained in the laboratory In the anechoic chamber the 6 subjects tested could vocalize on the average with a 6 dB lower intensity (range 0–14 dB) than in the laboratory

The reflex thresholds for contralateral acoustic stimulation were considerably higher (9–65 dB average, 36 dB) than the thresholds for stapedius activity produced by the subject s own vocalization The maximum EMG amplitude during acoustic stimulation of the 5 subjects with eardrum perforation varies between 55 and 153 % of that obtained during maximum vocalization, with an average value in the two conditions being nearly equal The high maximal value during acoustic stimulation for th subjects G O and V B can probably be ex plained by the fact that they did not use the full vocal capacity in the laboratory Th postulation is supported by the observation tha they could raise their maximum vocal intensit in the anechoic chamber at a later occasio (subject G O to 122 and subject V B to 11 dB SPL) The low maximum value of subjec B S most certainly was caused by the eardrun perforation and concomitant conductive hear ing impairment in the stimulus ear The refle threshold upon acoustic stimulation was there fore high (Table I) and the reflex response wa not saturated within the highest intensity ava able (130 dB SPL)

At a vocal intensity of 80 dB SPL 20 cm i front of the mouth which approximately cor responds to normal vocal effort (Fletcher, 195 p 68) the stapedius activity (as a percentage o maximum during vocalization) was between 3 (subject A H) and 80 % (subject B S) with a average of 52 Corresponding values in relatio

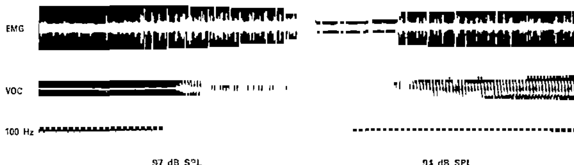


Fig 5 Temporal relation between EMG (upper trace) and speech signal (lower trace) at two vocal intensity levels Subject G O

to maximum during acoustic stimulation were 20% (subject G O) and 150% (subject B S) with an average of 61 Consequently the stapedius contraction is likely to have a significant influence on sound transmission during normal speech

Temporal relation between start of EMG and vocal sound

In order to study whether the stapedius muscle is activated during vocalization as an acoustic reflex only or co-activated in some other way as well, it is crucial to determine the exact beginning of the EMG in relation to the vocal sound Fig 5 shows the initiation of the EMG signal and the vocal sound in one subject at two nearly equal vocal intensity levels It is seen that the motor unit potentials appear before the start of the sound in one recording In the other recording the EMG lags behind the sound In all the subjects the EMG was observed to start sometimes before and sometimes after the start of the sound There was no apparent correlation between the vocal intensity and the temporal interval from signal to signal In 30 of the 55 vocalizations of [a] where the recordings could be interpreted exactly, the EMG appeared before the sound up to 150 msec In 27 of these recordings (90%) the first motor unit potential appeared 75 msec or less before the start of the sound The shapes of the motor unit potentials appearing before start of the sounds were also analysed with a faster sweep speed and compared with motor unit potentials in response to acoustic stimulation of the same subject (Fig 1) It is seen that the motor unit potentials appearing before as well as during vocalization and during acoustic stimulation are very similar with respect to shape and duration and differ significantly from those seen during teeth clenching, for instance Since the stapedius muscle can be activated before the start of speech sound it is concluded that the activation stems from the central nervous system as a part of the vocalization act

DISCUSSION

Mechanisms for stapedius muscle activation during vocalization

The present results showed that the EMG of the stapedius muscle during vocalization often appeared before the speech sound This result is in agreement with Henson's (1965) findings in some bat species and with the work of Carmel & Starr (1963) in the cat Thus, it is evident that the stapedius muscle can be activated non-acoustically both in animals and man as a part of the vocalization process Furthermore, in the present study in man it was found that the sta-

pedius muscle is active largely throughout the whole range of vocal intensities

The voice can be raised to levels well above 100 dB SPL as measured 20 cm in front of the mouth (one subject reached 122 dB SPL) For an [a] the sound pressure at the entrance of the auditory canal is almost the same as 20 cm in front of the mouth (Sundberg, personal communication) It may be concluded that the acoustic reflex activation plays a role at high voice levels but at low and medium voice levels the situation is more ambiguous

In the present material it was found that the stapedius muscle was activated during vocalization at a voice level (20 cm in front of the mouth) on the average 36 dB below the acoustic reflex thresholds for contralateral sound stimulation In 2 of the subjects the threshold during vocalization was as much as 65 (subject M G) and 53 dB (subject B S) below the acoustic reflex threshold One of them (subject B S) had a bilateral conductive hearing impairment with the lowest hearing threshold at 45 dB SPL (2 0 kHz) This value was only 15 dB below the threshold for stapedius activation during vocalization (see Table I) Thus the conductive g impairment in subject B S did not seem influence the stapedius activity during the s own speech while the stapedius reflex threshold for contralateral sound stimulation was quite strongly affected The other subject (M G) had hearing thresholds in both ears within 15 dB (HL, ISO 1964) in the range 0 5–2 0 kHz Since the acoustic reflex thresholds were far above the thresholds for stapedius activation during vocalization it is concluded that air-conducted sound played little part in the activation of the stapedius muscle during vocalization in these 2 subjects The results obtained from the other subjects also support the assumption that air-conducted sounds do not activate the stapedius muscle during at least low and medium intensity levels of a person s own voice

Neither the present results nor other data in the literature (Bekesy, 1960, p 181 Tonndorf, 1972, Djupesland et al , 1973) provide a basis for estimating the role of bone conducted sound for activation of the stapedius muscle during the subject's own vocalization

Functional significance of stapedius activity during vocalization

Discrimination of other persons' speech sounds while the subject himself is not talking has been found to be significantly better in ears with normal stapedius reflex than in ears with paralysed stapedius muscle (Borg & Zakrisson, 1973) This difference was most likely due to the attenuation of the low frequency components of speech sounds provided by the normal stapedius reflex The reduced masking (antimasking) on the high frequency speech components might thereby have been considerable (Borg & Zakrisson, 1974)

During vocalization the sound reaches the ear both by air- and bone conduction, the quantitative relation of which is not clear It has recently been shown (Irvine & Wester, 1973) that stapedius muscle contraction attenuates bone-conducted sound to about the same extent as it attenuates air-conducted sound The sound level at the ear due to the subject's own voice is high, especially for low frequency components of the produced speech (Fletcher, 1953 p 68, Fant, 1960 p 125) These low frequency components might mask high frequency speech sounds that simultaneously have to be discriminated The antimasking of the stapedius reflex is pronounced and may thus play a part in this connection When reflex activity is near maximum the masking of low frequency noise on high frequency tones decreases by as much as 50 dB (Borg & Zakrisson, 1974) Since the results of the present study show that the stapedius muscle is active to 50–60 % of maximum even at normal vocal effort, the antimasking can be expected to be significant also at this voice level The attenuation of the low frequency components of the subject's own speech sounds provided by the stapedius reflex therefore most likely improves intelligibility of simultaneous external speech

A correlate to this control function of the

stapedius muscle is found in the lateral-line organ of the dogfish which is sensitive to displacement of the water around the animal Roberts & Russell (1972) showed that the efferent nerve fibres to the hair cells were activated during the animal's own movements By blockage of the afferent neuronal activity from the hair cells, the sense organ could be prevented from being overstimulated and could maintain its sensitivity A similar efferent control of the sensitivity of the vestibular organ during movement in the goldfish was found by Klinke (1970) These three control functions thus seem analogous they decrease the influence of own activities in man and animal, e g vocalization and movement, that would otherwise reduce the receptive ability of the sensory organ

ZUSAMMENFASSUNG

An Patienten mit Trommelfellperforation wurden bei Eigenproduktion des Vokals a und bei akustischer Stimulation mit sin Tonen von 0 5 und 2 kHz die EMGs des Stapediusmuskels aufgenommen und die Bewegungen der Stapediussehne mikroskopisch beobachtet Die Schwelle der Stapediusaktivitat lag gerade uber der niedrigsten mit gewohnlicher Stimme aussprechbaren Lautstarke des Vokals a Bei angenehmer Lautstarke erreichte der Stapediusmuskel etwa 50% seiner maximal moglichen Aktivierung Die EMG Aktivitat setzte oft vor dem Beginn des Vokallauts ein, woraus zu ersehen ist dass die Stapediusaktivität nicht durch den Laut als solchen reflexartig ausgelost wird Die Untersuchungen legen des weiteren den Schluss nahe, dass die Kontraktion des Stapediusmuskels wahrend der eigenen Vokalproduktion die Verstandlichkeit gleichzeitigen Sprechens anderer Personen durch Minderung des Verdeckungseffekts verbessert, der durch niederfrequente Komponenten der eigenen Stimme bewirkt wird

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TYMPANIC MUSCLE REFLEX ELICITED BY ELECTRIC STIMULATION OF THE TONGUE IN NORMAL AND PATHOLOGICAL SUBJECTS

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Abstract A bilateral reflex contraction of the tensor tympani muscle has been obtained in man by electric stimulation of the tongue (1–2 mA) The stimulus is well tolerated and always effective The advantage is stressed of eliciting a contraction of this muscle without involvement of the stapedius as occurs with other methods An analysis has been subsequently conducted in normal subjects and in patients affected by pathology of the tympano-ossicular system tympanosclerosis otosclerosis suprastapedial facial paralysis in cases of interruption of the afferent arch section of the homo lateral lingual nerve in cases of involvement of its central portion cerebello-pontine angle tumours and in cases of section of chorda tympani A chiasm like central nervous pattern is suggested

increasing number of data demonstrate the reflex contraction of the tensor tympani scle can be elicited by tactile stimulation of some facial areas puff of air against the orbit (Klockhoff & Anderson, 1960) eyelid lifting (Djupesland, 1964), or by strong sound stimuli (Liden et al 1970) It can also be observed during the motor activity of some muscular groups of the face, of the neck and of the phonatory muscles (Salomon & Starr, 1963) In all these conditions however, the contraction of this muscle is not isolated but is associated with that of the stapedius being a sort of startle reaction to sudden stimuli, or correlated with the contraction of a more general muscular system (Kato, 1913 Klockhoff, 1960 Lindstrom & Liden, 1964)

However, the advantage of obtaining an isolated tensor tympani muscle contraction is obvious when studying the physiopathological conditions of its nervous reflex arc or of the middle ear structures, as is currently done with the stapedius muscle reflex To this end we have investigated the possibility of eliciting an isolated tensor tympani muscle reflex (t t m r) contraction by electric stimulation of the tongue, which is an organ furnished of a rich, sensitive innervation by the trigeminal nerve

MATERIAL AND METHOD

The stimulus was given by a positive and/or negative electrode (28 mm²) applied to one side of the tongue slightly protruded by the subject himself The organ was slightly moistened by saliva during the brief duration of the test The stimulus was of an intensity of 1–2 mA and about 1 sec in duration

During the stimulation the subject was pressing one wide-open hand on the ground plate To our experience, the large surface of this plate allows the application of these rather intense stimuli without unpleasant sensations or reactions from the subject Electrogustometry indeed, is performed by applying electric stimuli in the range from 3 to 300 μA The reflex was recorded by an Impedance Meter (Madsen ZO 70) connected to a graphic recorder (Minigor RE 501) and to an Oscilloscope (Tektronix 5103N/D13) with the probe inserted into the homolateral or contralateral ear canal The test was carried out in a quiet room

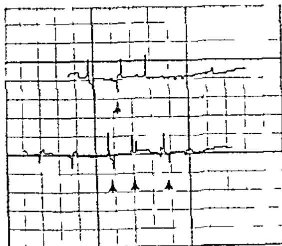


Fig 1 Graphic records of tensor tympani muscle reflex contraction in a normal subject Some of the contractions are indicated by an arrow

RESULTS

Normal Subjects

In a preliminary investigation (Bosatra & Russolo, 1973) 150 normal individuals, from 20 to 56 years old, were tested In all subjects the application of the stimulus provoked a bilateral reflex contraction of the t t m at the opening, but often also at the closure of the electric circuit, so that the response was characterized by a double spike with an interval as long as the duration of the stimulus (about 1 sec) The spikes were at random, positive or negative, relative to the base line (Figs 1 and 2) The explanation of this phenomenon is not easy at the moment the positive spikes are due to an increase in intensity of the sound wave emitted by the probe of the impedance meter, and this increase is due to a reduction in size of the meatal chamber because of an outward displacement of the tympanic membrane The opposite is true for the negative spikes

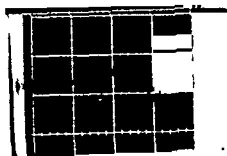


Fig 2 Oscilloscopic tracing of single tensor tympani muscle reflex contraction First spike at the closure second spike at the opening of the electric circuit

Thus, the opposite directions of the spikes may be due to the opposite mechanical effect at the level of the tympanic membrane of the very short and small contractions of this muscle which exerts a traction at nearly right angles to the chain, with a rotatory component

Pathological Subjects

In the present study, in addition to many more normal individuals, pathological ears have also been tested

(A) *Pathology of tympano ossicular system and related structures*

(i) 12 cases of otosclerosis were tested the traces obtained appeared to be normal or slightly reduced
(ii) 15 subjects showing a typical picture of tympanosclerosis with scarred and retracted drum, but with normal tubal function and presence of the stapedius reflex elicited by contralateral acustic stimulus, showed very reduced or no responses
(iii) 1 subject with complete bilateral velar paralysis, with atrophy of this organ due to previous diphtheria, showed slower responses (Fig 3)
(iv) 18 subjects with unilateral paresis of the VII nerve showed normal responses on the affected side
(v) 3 subjects with unilateral complete supra stapedial paralysis of the VII nerve showed greater than normal responses on the affected side (Fig 4)

(B) *Pathology of nervous arc*

(i) 2 cases were tested in which a unilateral surgical section of the lingual nerve at the level of the submandibular gland was

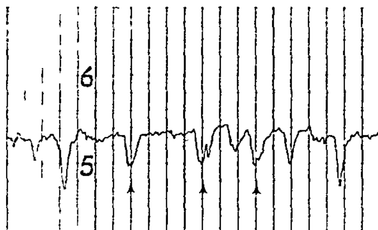


Fig 3 Graphic record of tensor tympani muscle reflex contraction in a case of complete velar paralysis Arrows indicate some of the contractions

performed during radical neck dissection the electric stimulation of the homolateral side of the tongue provoked no t t m r contraction bilaterally The response was present with the stimulus applied on the opposite side of the tongue, as it was present bilaterally previous to the operation

(ii) 4 cases were tested showing the clinical picture of a unilateral ponto cerebellar angle tumour, subsequently confirmed surgically and provoked by an acoustic neuroma In 3 cases the t t m r reflex was absent homolaterally to the side of the lesion for a stimulus applied homolaterally, whereas, the contralateral reflex was present With a contralateral stimulus, the t t m r was present bilaterally In these subjects the neurological picture also showed homolateral severe perceptive deafness, no or very reduced vestibular responses, spontaneous nystagmus to the homolateral and contra lateral side, homolateral severe hypogeusia to electrogustometry no other signs of V nerve involvement In the 4th patient, showing more severe clinical signs of brain stem involvement, with vertical nystagmus, reduced corneal reflex and facial palsy, the t t m r was bilaterally absent for an homolateral stimulus whereas the contra lateral stimulus elicited a bilateral con traction

(iii) We have not yet met with subjects with complete and strictly limited interruption of the motor portion of the V nerve

(iv) 13 cases have been examined in which a unilateral section of chorda tympani was performed for the treatment of facial palsy or during myringoplasty or stapedectomy In these cases the homolateral stimulation showed normal responses in the contra lateral ear and also in the homolateral one in all cases in which the anatomical con ditions of the tympano ossicular system rendered it possible

CONCLUSIONS

We believe that unilateral electric stimulation of the tongue with intensities of 1–2 mA is an adequate stimulus to elicit an isolated bilateral t t m r contraction, without involvement of the stapedius Indeed it is possible to observe the t t m r contraction elicited by this method superimposed on the maximal stapedial contrac tion elicited by sound stimuli

In our experience this method is always effective, whereas a puff of air directed towards the eye sometimes gives no response Moreover it is often unpleasant and when effective it is usually associated with a contraction of the stapedius muscle

By considering its shape, the t t m r contrac tion does not seem to be involved with the contraction of other muscular groups of the face and oropharynx as demonstrated especially

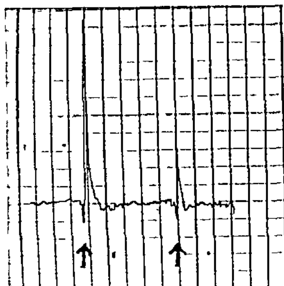


Fig 4 Graphic record of single tensor tympani muscle reflex contraction in a case of suprastapedial facial paralysis Arrows indicate the spikes at the closure and at the opening of the circuit

by the cases of unilateral central block of the reflex arch

The recording of this contraction by an impedance meter demonstrates that its me chanical effect at the level of the tympano

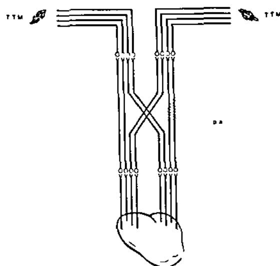


Fig 5 Schematic pattern of nervous arch of tensor tympani muscle (T T M) reflex *T* tongue Triangle marked c p.a t represents various sizes of cerebello-pontine-angle tumours surgically confirmed interfering with reflex pathway

ossicular system is small as a consequence, we believe, of the small intensity of the stimulus and of the physiological mode of action of this muscle

Obviously the size of the response is related to the conditions of the mechanical effector it is greater in cases of complete facial paralysis or interruption of the ossicular chain It is smaller or abolished in cases of stapes anchylosis or tympanosclerosis

The t t m r is absent when the stimulus is homolaterally applied to the section of the lingual nerve It is not affected by the section of chorda tympani The bilateral distribution of the reflex seems to follow a chiasm like nervous pattern, as demonstrated in cases of brainstem involvement due to unilateral ponto-cerebellar angle tumour (Fig 5)

RESUME

Une contraction reflexe bilaterale du muscle tensor tympani a éte obtenue sur l homme par stimulation électrique de la langue (1 2 mA) Le stimulus est bien tolere et toujours efficace L avantage est que l on obtient une contraction de ce muscle sans celle du stapedius comme il arrivait avec d autres methodes Ensuite une analyse a eté effectuee sur des sujets normaux et sur des pat ents atteints de lesions du systeme tympano-ossiculaire Tympanosclerose otospong ose paralysie faciale suprastapedienne en cas d interruption de l arc afferent section du nerf lingual homolatéral en cas d at teinte dans sa portion centrale tumeurs de l angle pontocerebelleux et en cas de section de la chorda tympani On envisage un croisement des voies nerveuses centrales

ZUSAMMENFASSUNG

Durch die elektrische Reizung der Zunge (1 ? mA) ist in der Paukenhohle des Menschen eine Reflexe beidseitige Kontraktion des Musculus Tensor Tympani erzielt worden Dieser Reiz ist gut vertraglich und ausreichend Der Vorte l der Methode liegt darin dass eine Kontraktion dieses Muskels hervorgerufen wird ohne dass der Steigbugel daran teiln mmt wie dies bei anderen Methoden der Fall ist Es ist daher eine Ver suchsreihe durchgefürt worden sowohl an gesunden als auch an Pat enten die am knochernen tympanischen System erkrankt waren und zwar an Tympanussklerose Gehorsklerosen Lähmung des Nervus Facialis oberhalb des Stapes in ein gen Fallen an Unterbrechung des zu fuhrenden Bogens Durchtrennung des Nervus Lingual s der gleichen Seite in einigen Fallen an Verletzung des zentralen Teiles des Nerven in e nigen Fällen an Unter

3 brechung der Corda Tympani Tumoren an der Brücke zum Gehirn Bei diesen Fallen ist an ein Überschneiden der zentralen Nervenbahnen zu denken

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THE EFFECT OF 6-HYDROXYDOPAMINE ON THE RABBIT COCHLEA

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Abstract 6-OH DA is an isomer of noradrenalin which is selectively taken up by adrenergic axons Noradrenalin stores are displaced by 6-OH DA and at a certain intraneuronal concentration degeneration of the terminals occurs and results in a chemical sympathectomy The effects of 6-OH DA on the rabbit cochlea were studied with fluorescence and electron microscopy after systemic administration of the substance and after local perfusion of the cochlea Doses of 25 to 200 mg/kg were used After intravenous injection there was an initial accumulation of 6-OH DA in noradrenalin storage vesicles A dose of 50 mg/kg 6-OH DA initiated marked signs of degeneration in adrenergic nerve terminals but did not cause breakdown of their cell membranes Higher doses did not seem to increase the damage Local perfusion with 6-OH DA gave rise to extensive degeneration of adrenergic nerve terminals and after 7 days all terminals had disappeared These findings indicate the presence of a blood perilymph barrier to 6-OH DA Some degeneration was also evident in cholinergic axons of the inner spiral bundle and of the tunnel spiral bundle

6 hydroxydopamine (6 OH-DA) is an isomer of noradrenalin (NA) and also of the false transmitter 5-hydroxydopamine (5-OH-DA) In some respects it behaves like a catecholamine Thus it has been shown to be taken up and accumulate in peripheral terminals of adrenergic neurons by the membrane pump mechanism (Jonsson & Sachs, 1970 Ljungdahl et al, 1971) 6-OH DA is known to cause a long lasting depletion of NA in sympathetically innervated organs (Porter et al, 1963, 1965 Laverty et al, 1965, Thoenen & Tranzer, 1968, Malmfors & Sachs, 1968) Initially, 6 OH-DA acts as a false transmitter and displaces the endogenous NA in the amine-containing granules When the concentration reaches a critical level, the catecholamine uptake and storage mechanisms are damaged and the terminals degenerate The exact mechanism behind this degeneration is not known As 6-OH-DA is readily oxidized at physiological pH, it has been suggested that the oxidation products of 6-OH-DA become covalent bound to certain biologically important macromolecules (Thoenen et al, 1970) In adult animals 6-OH-DA produces an acute and specific degeneration of adrenergic nerve endings, i e chemical sympathectomy (Tranzer & Thoenen, 1967, 1968, Tranzer & Richards, 1971, Heikkila & Cohen, 1971)

Administration of 6-OH-DA *in vivo* results in effects of a varying extent in different organs different neurons in the same organ may also be affected differently It has been suggested that regional differences in vascularization cause these differences in the 6-OH DA concentration (Malmfors & Sachs, 1968, Jonsson & Sachs, 1970) The ultrastructural and functional effects of 6-OH-DA on sensory organs have not yet been investigated Compared with other species the rabbit cochlea receives a particularly rich adrenergic innervation, which is especially concentrated to the demyelinization zone of the axons and the spiral vessel of the tympanic lip (Densert, 1974) Thus the rabbit is well suited for investigations on the morphology of the cochlear adrenergic innervation as well as functional aspects of the sympathetic influence

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on sound perception The aim of this investigation was to study the effects of 6-OH-DA on the rabbit cochlea after systemic and local administration

MATERIAL AND METHODS

In this study 28 white rabbits weighing from 1 0 to 3 5 kg were used

Intravenous administration

In 20 animals a solution containing 10 mg/ml 6-OH-DA-HCL (2,4,5-trihydroxyphenethylamine hydrochloride, H 88/32, Hassle) and 0 2 mg/ml ascorbic acid was injected via an external ear vein

In 13 animals 50 mg/kg 6-OH-DA in the form of the above solution was administered as a single injection For electron microscopy, decapitation under Nembutal® anaesthesia was performed after ½, 1, 2, 6, 12 hours and 3, 7 days respectively For fluorescence microscopy, 5 animals were used and sacrifice was performed after 3, 6, 24 hours and 7 days respectively One animal was used as a control

In 5 animals 25, 100 and 200 mg/kg 6-OH-DA as administered to investigate the ultrastruc- al effects of low and high doses Two animals e given repeated doses of 2×25 and 2×50 mg/kg at 2 hour intervals and they were sacrificed after 3 or 7 days

Electron microscopy The specimens were fixed in cold veronal acetate buffered 1% osmium tetroxide (Rhodin, 1954), dehydrated in ethanol and embedded in Epon (Luft, 1961) Ultrathin sections were cut with diamond knives on an LKB ultrotome The sections were stained in uranyl acetate (Reynolds, 1963) and lead citrate (Watson, 1958) and were examined in a Siemens Elmiskop I or Philips 300 electron microscope

Fluorescence microscopy The cochlear turns were dissected out and mounted on glasses as stretch preparations and dried for 1 hour *in vacuo* The specimens were treated with formaldehyde gas according to the method of Falck & Hillarp (for ref see Corrodi & Jonsson, 1967) After mounting in Entellan®, the specimens were examined in a Zeiss epi fluorescence microscope

Local perfusion

In 5 animals the tympanic bulla was opened and with the aid of a diamond drill a small hole was made to the scala tympani The stapes was detached and the cochlea was perfused via the drilled hole for 5 minutes with a solution containing 0 2 mg/ml ascorbic acid and 0 1 mg/ml 6 OH DA The cochlea was then perfused with only Ringer solution and 3 animals were left for about 10 minutes, one for 24 hours and one for 7 days before sacrifice As controls in 2 animals the cochlea was perfused with the above-mentioned solution but 6-OH-DA was excluded The control animals were sacrificed after 10 minutes and 7 days respectively

Electron microscopy was performed according to the procedure described above

RESULTS

Intravenous administration

Interest was concentrated to the area where the cochlear adrenergic innervation was most pronounced, i e around the spiral vessel of the tympanic lip and the habenula region where axons were losing their myelin sheaths (Fig 1)

When 50 mg/kg 6 OH-DA was administered as a single i v injection, within 30 minutes a black core appeared indicating there was an accumulation of the substance in synaptic vesicles Definite signs of degeneration could already be observed at this time During the first 3 hours at least 2/3 of the terminals showed signs of degeneration Mitochondria were transformed to homogeneous contourless masses (Figs 2 and 3)

There was a loss of vesicles and some terminals contained only debris and amorphous clumps The axonal cell membranes did not show any signs of rupture or disintegration

There was no difference in the degree of degeneration or in the accumulation of 6-OH DA in terminals located around blood vessels or in the habenula region In fluorescence microscopy


B
μ

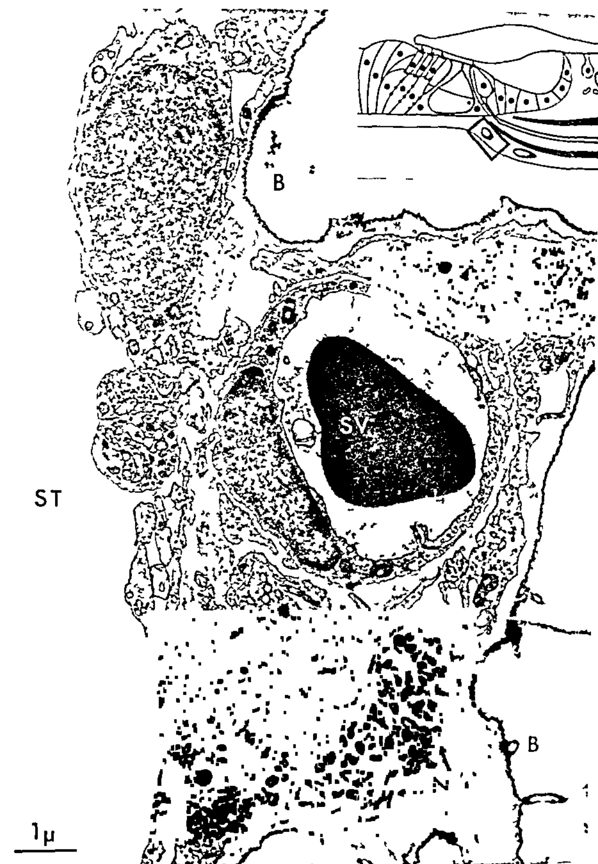

B
SV
ST
B
N
1 µ



Fig 3 High magnification from part of Fig. 2 The adrenergic nerve terminals (*NT*) contain mitochondria in various stages of degeneration and the number of synaptic vesicles is markedly reduced Less affected terminals contain vesicles with a dense core (*) indicating accumulated 6-OH DA No Schwann cells are observed 34 300

Fig 2 Spiral vessel of the tympanic lip (*SV*) surrounded by damaged adrenergic nerve terminals (*NT*) Most terminals contain mitochondria in different stages of degeneration One hour after i v administration of 50 mg/kg 6-OH DA *B* osseous spiral lamina, *ST* scala tympani See Fig 3 × 13 500

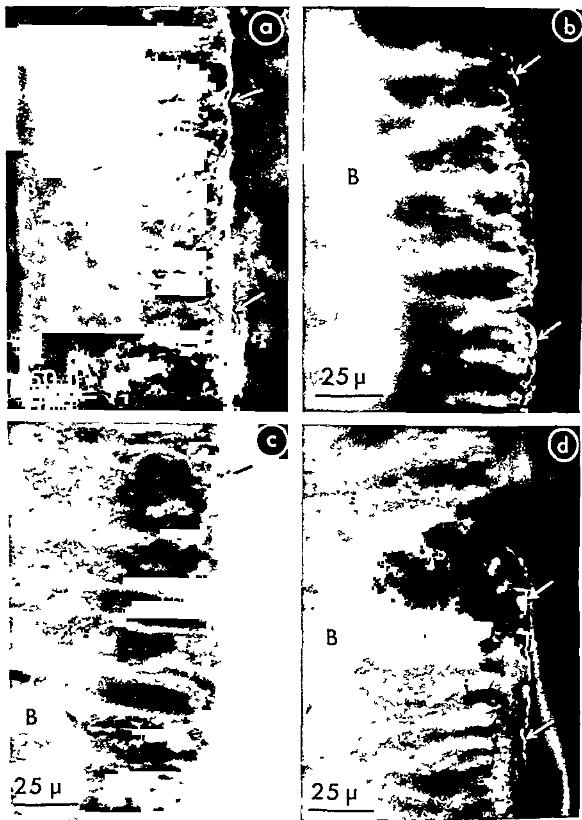


Fig 4 The effect of 6-OH DA on the fluorescence of adrenergic fibres in the habenula region Surface preparations from the second coil (*a*) Untreated rabbit 400 (*b*) Six hours after i v administration of 6-OH DA the fluorescence is markedly reduced ×630 (*c*) Twenty fou hours after the injection practically all fluorescence ha disappeared ×630 (*d*) Seven days after the injectio fluorescence starts to reappear ×630 *B*, bone

the fluorescence in the cochlea was slightly reduced after 3 hours

After 6 hours the content of some terminals was totally disintegrated and only debris was left inside the terminals Severely affected terminals still contained small vesicles with a dense core At this time most terminals seemed to be markedly affected, although some terminals with normal mitochondria, a few dense-cored vesicles and only a few disintegrated synaptic vesicles were also observed The fluorescence was strongly reduced and was now graded along the coil, it had totally disappeared in the apical coil, whereas a faint fluorescence remained in the rest of the cochlea (Figs 4*a*, *b*)

After 12 hours most adrenergic nerve terminals appeared practically normal (Figs 1 and 5) Some terminals showed signs of total disintegration They contained amorphous masses or just debris In large synaptic vesicles a black dense core was still present while this was seldom the case in small vesicles (Fig 5) The degeneration did not progress after one day Degenerated mitochondria were found in some terminals and in the vesicles no signs of 6-OH-DA remained After one day, only a few fluorescent fibres were detectable in the entire cochlea (Fig 4*c*)

Ultrastructural changes were still present after 7 days A large number of terminals were of normal appearance and the number of terminals also seemed to be the same as in untreated animals Totally disintegrated terminals were no longer seen Furthermore, fluorescence started to reappear, beginning in the second coil (Fig 4*d*)

Administration of a lower dose (25 mg/kg) of 6-OH DA resulted in an accumulation, mainly in large storage vesicles, but produced no ultrastructural signs of degeneration 25 – 25 mg/kg resulted in very discrete changes in adrenergic nerve terminals and only a few terminals contained amorphous mitochondria, synaptic vesicles appeared normal Increasing the amounts of 6-OH DA (50 + 50, 100 and 200 mg/kg) did not seem to induce more pronounced damage than that caused by 50 mg/kg administered as a single dose

Local perfusion

When the cochlea was perfused locally with a 6-OH-DA solution, 6-OH-DA seemed to readily penetrate the membranous walls of the cochlear partition, since it accumulated to the same extent in terminals around the spiral vessel of the tympanic lip and in terminals in the habenula region Even 10 minutes after the perfusion practically all synaptic vesicles contained an osmophilic core and large synaptic vesicles were almost filled with this osmophilic substance (Fig 6) Degeneration had already started and in most terminals several small vesicles had disintegrated, leaving in their place only a moderately electron dense material Mitochondria showed a varying degree of degeneration and in many terminals their remnants were represented by amorphous masses The membranes of the terminals were intact and did not show any signs of degeneration or rupture After one day it was impossible to identify any normal adrenergic nerve terminals There was a total disintegration of the terminals and at the site where they were normally easy to find, around the spiral vessel of the tympanic lip, only dark debris remained After 7 days, all traces of adrenergic terminals had vanished The organ of Corti now showed marked signs of degeneration, evidently due to the mechanical trauma that occurred when the cochlea was perfused

Efferent nerve terminals

In efferent nerve terminals small synaptic vesicles appeared empty, while large vesicles had a weakly stained content There were no ultrastructural signs of accumulation of 6-OH-DA However, even 2 hours after the administration of 6-OH-DA, homogenous bodies resembling the degeneration products in adrenergic nerve terminals were observed in a small number of efferent nerve fibres (Fig 7) It was most evident in the tunnel spiral bundle where amorphous clumps of variable sizes appeared in almost every electron microscopic section The same kind of degeneration also appeared in

terminals of the inner spiral bundle and below the inner hair cell (Figs 1 and 8)

The injuries described above in efferent axons appeared when 50 mg/kg 6-OH-DA was administered i v and did not seem to become more pronounced when this dose was exceeded Local perfusion with 6-OH DA did not seem to aggravate the damage

Apart from the probable mechanical injury to the cochlea following local perfusion, inner and outer hair cells, like afferent nerve fibres, remained unaffected after both local and systemic administration of 6-OH-DA

DISCUSSION

6-OH-DA is known to interfere with the function of the sympathetic nervous system It causes a long lasting depletion of noradrenalin in peripheral adrenergic neurons (Porter et al, 1963, Laverty et al, 1965) It was shown by Tranzer & Thoenen (1967, 1968), in electron microscopy, that this effect was due to a rapid accumulation of the substance in storage vesicles Subsequent studies have indicated a rapid destruction of adrenergic nerve terminals (Bennett et al, 1970, Tranzer & Richards, 1971)

6-OH DA readily passes through the axonal membrane, carried by the amine pump mechanisms It is possible to inhibit this uptake mechanism with desmethylimipramine (DMI) and thus prevent destruction of the terminals (Stone et al, 1964) Reserpine, which acts on the storage mechanism of the vesicles does not prevent destruction of the terminals (Malmfors & Sachs, 1968) It is therefore assumed that accumulation of 6-OH-DA in synaptic vesicles is not the cause of terminal destruction, which occurs when the concentration of 6-OH DA or its metabolites in the axon exceeds a critical level

The cochlea has a rich vascular and adrenergic nerve supply, the adrenergic innervation being particularly prominent in the rabbit cochlea (Densert, 1974) From the results of this investigation it does not seem possible to obtain a complete sympathectomy in the cochlea after i v administration of 6 OH-DA Ultrastructural changes occurred rapidly but within 6 hours the degeneration had reached a maximum and did not progress further There was no difference in the degree of damage between terminals located around the spiral vessel and those in the habenula region In fluorescence microscopy, the fluorescence did not disappear completely after 50 mg/kg 6-OH-DA and started to increase again within 7 days

Adrenergic nerve endings regenerate after treatment with 6 OH-DA (Häusler, 1971) Functional recovery is more rapid than recovery of NA levels might indicate and may be registered when as little as 25% of the normal NA content is restored *In vivo* a marked difference has been demonstrated in sensitivity to 6-OH-DA between species different organs and even between neurons in the same organ (Malmfors, 1971)

It was interesting to note that some adrenergic terminals seemed quite unaffected by 6-OH DA while neighbouring ones showed marked ultra-structural signs [illegible]

Fig 5 High magnification from part of Fig. 1 Some terminals show signs of degeneration (↑) while others appear practically normal Twelve hours after the administration of 6-OH DA A few dense-cored vesicles still remain (↕) *SV*, spiral vessel of the tympanic lip × 30 000

Fig 6 Section from the habenula region Ten minutes after local perfusion of the cochlea Note the heavy accumulation of 6-OH DA in adrenergic synaptic vesicles with signs of degeneration and disintegration *NT*, adrenergic nerve terminal 37 000

7
0.5 µ
8
ISB
D
fig.8
ISB
D
P

In this respect there is no difference between terminals associated with blood vessels and with afferent nerve fibres

In earlier investigations (Tranzer & Thoenen, 1968), it has been shown that the Schwann cell seems to engulf severely damaged terminals It might be concluded that the relatively limited sensitivity of cochlear adrenergic axons to i v administration of 6-OH DA was due to this paucity of Schwann cells However, it is more likely that this is instead related to the function of the capillary walls as barriers between plasma and perilymph

There is a difference in the susceptibility of damage depending on the route of administration, the degree of damage is much greater and it appears much faster after local perfusion of the scala tympani than after i v administration This is in agreement with results from other investigations which indicate a separation of capillary space and scala tympani Thus Galey et al (1971) found that i v injection of Flaxedil, which is a blocking agent for cholinergic synapses, is without effect on the inhibition caused by stimulating the crossed olivo-cochlear bundle However, local perfusion of the scala tympani with Flaxedil blocks this inhibition

Duvall et al (1971) have used horseradish peroxidase as a tracer of communication between inner ear fluid spaces They found that the extracellular space of the habenula region and the scala tympani are in free communication and are separate from the lumen of the cochlear blood vessels

It should be noted that following 6-OH DA administration no changes are observed in hair cells or at their afferent synapses, especially since a monoamine has been suggested to be the afferent transmitter (Osborne & Thornhill, 1972) It is known from several investigations that 6-OH DA is highly specific and acts selectively on adrenergic nerve terminals (Tranzer & Thoenen, 1968, Bennett et al, 1970, Furness et al, 1970, Siggins & Bloom, 1970) Efferent nerve fibres in the inner ear are believed to be cholinergic in nature (see Fex, 1973) It was therefore surprising to find that the efferent axons showed signs of degeneration The damage was restricted to the inner spiral bundle, tunnel spiral bundle and tunnel crossing fibres In these efferent axons the same kind of electron dense clumps, evidently degenerated mitochondria, appeared Some nerve fibres seemed to be damaged and had a swollen appearance, with reduced intracellular content Sometimes it was difficult to judge whether the observed changes were artefacts or represented signs of true degeneration It is known that the adrenergic axonal cell membrane is damaged by 6-OH-DA and that the amine pump mechanisms are inhibited

The possible effects on cholinergic axons may occur when the concentration of 6-OH DA in blood plasma is high enough One may also speculate on an initial concentration of the substance in adrenergic nerve terminals followed by a leakage of 6-OH DA and/or its metabolites in amounts large enough to have a toxic influence on cholinergic nerve fibres

If these findings are generally applicable the toxic effect of 6-OH DA will not be restricted to adrenergic axons but will also occur in other tissues if the concentration is sufficiently high In most organs adrenergic and cholinergic axons occur together, which makes it difficult to detect changes in cholinergic fibres In recent studies the possibility of a nonspecific action of 6-OH DA was reported by Poirier et al (1972), Hokfelt and Ungerstedt (1973)

Administration of 6-OH DA to rabbits in doses as high as 200 mg/kg produces ultrastructural damage in adrenergic axons but some axons still remain and may be functional The use of 6-OH DA to produce sympathectomy in physiological or pharmacological experiments must therefore be regarded as unreliable, at least if it is administered i v Furthermore, 6-OH DA seems to induce injuries also in some

Fig 7 Section from the tunnel spiral bundle The same kind of degenerating mitochondria as in adrenergic nerve terminals are found in some efferent axons. Degenerated mitochondria (*D*) 30 900

Fig 8 Section from the inner spiral bundle (*ISB*) with degenerated mitochondria (*D*) *P* inner pillar cell *TSB* tunnel spiral bundle 12 300

efferent axons, at least in the cochlea Surgical sympathectomy is therefore to be preferred as being more selective and reliable

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Åke Flock [illegible]
guidance an [illegible]
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ZUSAMMENFASSUNG

6-OH DA ist ein Isomer von Noradrenalin welches von adrenergen Axonen selektiv aufgenommen wird Bei einer bestimmte Konzentration kommt es zur Degeneration der Nervenendigungen und daraus resultiert eine chemische Sympathektomie

Die Wirkung von 6-OH DA auf die Kaninchencochlea wurde nach allgemeiner Verabreichung der Substanz und nach lokaler Durchstromung der Cochlea mit dem Fluoreszenz und dem Elektronenmikroskop untersucht Die verwendete Dosis betrug 25–200 mg/kg. Nach intravenoser Injektion erfolgte eine initiale Anhäufung von 6-OH DA in Noradrenalin Speichelvesikeln. Eine Dosis von 50 mg/kg 6-OH DA bewirkte deutliche Degenerationserscheinungen in den adrenergen Nervenendigungen, verursachte jedoch keine Zerstorung ihrer Zellmembranen. ohere Dosen schienen die schadigende Wirkung nicht verstarken Lokale Durchstromung mit 6-OH DA e zu umfangreicher Degeneration der adrenergen ervenendigungen und nach 7 Tagen zu einem Schwund er Nervenendigungen. Diese Beobachtungen geben Hinweise auf eine Blut Perilymph Schranke fur 6-OH DA Degenerationszeichen wurden auch an efferenten Axonen des inneren Spiralbundels und des Tunnel spiralbundels beobachtet.

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THE VASCULAR PATTERN OF THE CHINCHILLA COCHLEA

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Abstract The vasculature of the chinchilla cochlea was demonstrated with injected Prussian blue contrast By and large the vascular pattern is similar to other mammals The vasculature appears to be richly developed but separate types of vessels appear to be of small caliber Particular findings for the chinchilla cochlea were the veins of the scala tympani formed by a merging of collecting venules running parallel to the spiral modiolar vein Furthermore no vessel of the basilar membrane under the tunnel of Corti was found, and which, when present is of such great presumed importance for the oxygen supply to the organ of Corti The scala vestibuli wall appears to be supplied completely arterially, in contrast with that of other mammals The stria vascularis is broad and well developed both at the apex and at basal end

chinchilla has recently become one of the common animals for cochlear research due to the ease with which it is behaviorly trained for hearing testing and due to the favorable accessibility of the middle and inner ear for electrophysiological measurements

A knowledge of the cochlear vascular supply belongs to the basic data needed for the understanding of physiological and pathological conditions We have not been able to find any information in current or older literature on the vascular supply to the chinchilla cochlea The aim of the present investigation was to examine the vascular pattern of the chinchilla cochlea Emphasis is laid on the particular features of the chinchilla cochlear vasculature in relation to other mammals (man, Rhesus monkey, rabbit, and guinea pig)

As is well known the chinchilla has mainly been domesticated because of its extremely soft fur It is a relatively docile, plentiful animal which is easy to handle The cochlea stands prominent in the middle ear cavity, giving ready access for electrophysiological and surgical manipulation The chinchilla is more sensitive to noise exposure than are many other mammals The chinchilla is susceptible to pneumonitis, intestinal infections, disorders of the teeth, fungal infections of the skin, and kidney problems It is very sensitive to temperature changes and any rapid changes often are complicated by pneumonia The most suitable room temperature is 65° to 70°F (18°–24 C) The gestation period of a chinchilla is 111 days, and the average of litters is two a year with two offsprings per litter At birth, the young animals are fully furred, have a full set of teeth open eyes, and can walk and climb the sides of the cage

MATERIAL AND METHODS

Ten healthy adult chinchillas of both sexes were used for the experiments Behavior measurements of the hearing according to a modification of the method advanced by Miller (1970) disclosed normal hearing in all animals The cochlear vasculature was demonstrated by a contrast injection method previously described in detail (Axelsson, 1968, 1972, Axelsson et al. 1974*a*) Under general anesthesia and with a transcardiac approach, a plastic catheter was introduced into the ascending aorta and ligated The animal was perfused with 60 to 100 ml physiological saline of body temperature at a static pressure of 70 cm in order to remove the

blood from the vessels After completing saline perfusion, the animal was perfused with 2 5% Prussian blue in water of similar pressure and temperature as the saline solution The perfusion was terminated when the contrast solution entered the vascular system very slowly

The temporal bones were immediately removed, the bulla opened, the stapes removed, and small openings made in the round window and the cochlear apex The cochlea was fixed in 5% glutaraldehyde injected through the oval and round windows and then immersed for 24 hours The cochlea was then decalcified either with a buffered 8% EDTA (pH 7 4) or with 5% nitric acid After decalcification, some cochleas were counterstained with 1% osmic acid adjusted with Millonig buffer (pH 6 5) Subsequently, the cochlea was washed, dehydrated in increasing concentrations of alcohol, and stored in glycerin The cochlear vasculature was studied under the stereomicroscope in dissected pieces Representative specimens were photographed with a photomicroscope (Wild M 20)

The nomenclature adopted for the cochlear vessels is based on the relation of vascular structures to other anatomical structures or on the course of the vessels in the cochlea The nomenclature, consequently, is not based on the histological structure of the vascular wall and may thus cause disagreement as to the naming of vessels An arteriole, for instance, is here looked upon as any vessel connecting a large supply with the terminal vascular bed, where the vessels are termed capillaries, regardless of their morphological structure In the same way, a collecting venule is considered as a vascular structure connecting the capillaries with a vein, etc

GENERAL FINDINGS

The chinchilla cochlea is situated in a very large bulla which is divided into a few separate compartments by very thin lamellae The cochlea consists of three complete turns, and is slender, conical in shape, and similar to that of the guinea pig

The injections turned out well and with a more complete and even injection than is achieved in the guinea pig (Fig 1) The amount of Prussian blue needed for the demonstration of the vasculature was 20 to 50 ml, with an average of 30 ml

Vascular Anatomy

Modiolus

The following vascular structures were regularly demonstrated in the modiolus

The spiral modiolar artery
The spiral modiolar vein
The veins of the scala tympani
Radiating arterioles
Collecting venules
Capillaries of the spiral ganglion
Capillaries of the acoustic nerve
Capillaries of the modiolus wall

The major vascular supply of the cochlea is very similar to that of the guinea pig and rabbit and is maintained by the spiral modiolar artery (SMA) situated at the level of the scala vestibuli in the modiolus (Figs 1, 2) The artery sometimes seems to ramify terminally in two or three large primary branches at the level of the second turn The venous drainage is achieved by one vein, the *spiral modiolar vein* (SMV), running spirally around the modiolus basal to the spiral ganglion and close to the acoustic nerve (Figs 1, 2) Many *radiating arterioles* (RAL) leave the spiral modiolar artery and its primary branches and ramify several times in the modiolus The occurrence of these radiating arterioles is more frequent than in the guinea pig, rabbit, monkey, and man The radiating arterioles have a pronounced serpentine course in the modiolus and in the central aspects of the scala vestibuli but do not form "spring coils" as in the guinea pig Separate branches are situated more closely together after ramifications than in the guinea pig The radiating arterioles supply the capillary areas in the external wall, spiral lamina, and modiolus The *collecting venules* (CVL) drain the capillary areas in the same regions, have a



Fig 1 Chinchilla cochlea Longitudinal paramodiolar section. The cochlea has three turns and is surrounded by a thin bone shell The vascularity of the cochlea is rich *SMA*, spiral modiolar artery, *SMV*, spiral modiolar vein, *RAL*, radiating arterioles.

...ter appearance, and gradually merge to ... collecting venules The CVL from the ... wall merge with those coming from the spiral lamina and modiolus (Fig 3) The collecting venules regularly merge to form veins, the *veins of the scala tympani*, which take a spiral course situated more peripherally and apically but parallel to the spiral modiolar vein into which they empty (Fig 3) This arrangement has not been observed in the other mammals investigated As in the other mammals, delicate *capillaries* form vascular nets in the region of the *spiral ganglion*, in the *acoustic nerve* (Fig 4), and in the *modiolus wall* without any regularly recurring pattern

Spiral lamina

The following structures can be identified in the spiral lamina (Fig 5)

Radiating arterioles
Collecting venules
The vessel of the tympanic lip (inner spiral vessel)
The limbus vessels
(The vessel of the basilar membrane) (vas spirale, outer spiral vessel)

The vascular supply to the spiral lamina is maintained by centripetally *radiating arterioles* (RAL) These have a straighter course and are of finer caliber than the corresponding vessels in the guinea pig Each RAL supplies a small segment of the spiral lamina As in the external wall, *ramifications of the RAL in the spiral lamina* are more frequent centrally than peripherally Anastomoses occur between separate branches of the radiating arterioles and between them and the collecting venules The radiating arterioles supply the spiral peripheral vascular margins situated in the tympanic lip, the *vessel of the tympanic lip*, and in the spiral limbus, the *limbus vessels* (Fig 5) Occasionally, the *vessel of the basilar membrane* (VSBM) (vas spirale, outer spiral vessel) can be found under the

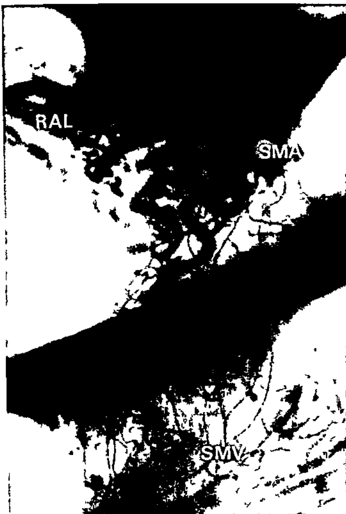


Fig 2 Chinchilla cochlea. Longitudinal section Modiolus The serpentine spiral modiolar artery (*SMA*) and central parts of the radiating arterioles (*RAL*) are seen at the level of the scala vestibuli The spiral modiolar vein (*SMV*) is situated deeper in the modiolus than in the other mammals

tunnel of Corti, most frequently in the third turn and only for short distances (Fig 5)

The vascular pattern of the tympanic lip is arcadic with a spirally running vascular margin which is more or less continuous the *vessel of the tympanic lip* (VSTL) (Figs 5 11) Supplying radiating arterioles and draining collecting venules join the vessel of the tympanic lip in a T formed or Y formed manner Occasionally, additional vascular arcades are formed both centrally and peripheral to the vessel of the tympanic lip The *limbus vessels* (LVS) form sparse loops with an irregular peripheral border in the spiral limbus (Fig 6)

The spiral vascular margins are drained by centripetally coursing *collecting venules* (CVL) parallel to the radiating arterioles Like the radiating arterioles, they are of finer caliber than the corresponding vessels in the guinea pig and rabbit The CVL merge to form larger collecting venules which finally empty into the veins of the scala tympani and the spiral modiolar vein The vestibular and tectorial membranes and the basilar membrane peripheral to the vessel of the tympanic lip are avascular, which fact is in agreement with the principal vascular pattern of the other mammals examined

External wall

The following vascular structures can be demonstrated in the external wall (Fig 7)



Fig 3 Chinchilla cochlea Longitudinal section Scala tympani basal turn Collecting venules (*CVL*) from the xternal wall and from the spiral lamina merge to form the vein of the scala tympani (*VST*) in the lower medial aspect of the scala Subsequently this vein empties into the spiral modiolar vein (*SMV*)

Scala vestibuli

adiating arterioles (suprastrial vessels)

Scala media

Stria vascularis
The vessel of the spiral prominence
Arteriovenous anastomoses

Scala tympani

The venules at the basilar membrane
Collecting venules

Scala vestibuli

The vascular supply to the scala vestibuli is completely arterial The *radiating arterioles* (RAL) run a serpentine apico-basal course before they straighten out Ramifications are most frequent in apical parts of the scala vestibuli Separate branches lie closer together after the point of ramification than in the other mammals investigated (Fig 8) In the apical parts of the external wall, the vascular wall of the radiating arterioles and the perivascular spaces surrounding them are easily demonstrated with phase contrast microscopy Contrary to the other mammals examined, no collecting venules can be found in the scala vestibuli wall of the chinchilla cochlea Likewise, there is no regularly occurring vessel *immediately above the attachment* of the vestibular membrane, nor is there a capillary net in this area Thus the scala vestibuli wall of the chinchilla appears to be completely arterial

Scala media

The external wall of the scala media is dominated by a very well formed *stria vascularis* (SVS) (Figs 7, 10) In the basal turn the SVS forms a capillary net which includes a breadth of up to ten loops of capillaries, whereas in the third turn this number has decreased to five The diameter of the capillaries in the

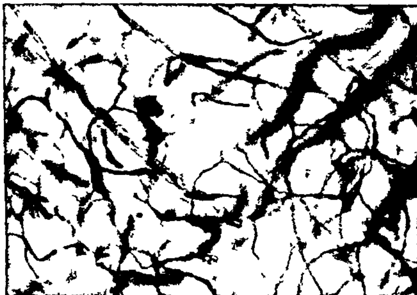


Fig 4 Chinchilla cochlea Spiral ganglion There is a net of dense delicate capillaries without any regular pattern

stria vascularis appears more delicate than in other mammals investigated Contrarily, the apical simplification encountered in other mammals is much less pronounced in the chinchilla The *vessel of the spiral prominence* (VSSP) is formed by short, spirally coursing parts of capillary vessels in the spiral prominence (Figs 7, 9) The VSSP is supplied by separate branches of the radiating arterioles in the scala vestibuli Like the stria vascularis the VSSP

Fig 5 Chinchilla cochlea Transverse section Spiral lamina, basal turn There is a spiral capillary arcade in the tympanic lip the vessel of the tympanic lip (*VSTL*) There is no regular vessel of the basilar membrane under the tunnel of Corti In the spiral limbus the limbus vessels (*LVS*) make up a spiral capillary arcade The capillary vessels are supplied by segmentally distributed radiating arterioles and drained by collecting venules *SGn* Spiral Ganglion

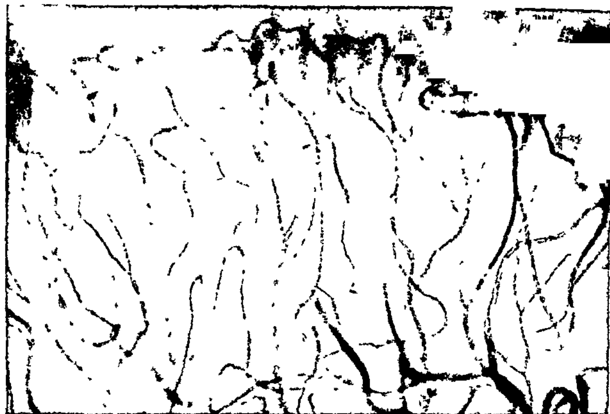


Fig 6 Chinchilla cochlea. Transverse section. Basal turn In the spiral limbus, irregular vascular arcades form the limbus vessels.

its separate arterial and venous supply It drained by many collecting venules turning in an omega-formed fashion around the attachment of the basilar membrane As in other mammals, branches from RAL and CVL are the only vessels connecting to the vessel of the spiral prominence There are no connections with capillaries in the stria vascularis The radiating arterioles supplying the vessel of the spiral prominence are more numerous than those supplying the stria vascularis

External to the stria vascularis and the vessel of the spiral prominence there are frequent *direct connections between the radiating arterioles* in the scala vestibuli and the collecting venules in the scala tympani They are here termed *arterio-venous anastomoses* (AVAS) They are most abundant in the basal turn and less frequently occur apically

Scala tympani

After an omega-formed turn around the attachment of the basilar membrane, the *collecting venules (CVL) often run a spiral course for some* distance before again turning basally These spiral parts of the collecting venules are here termed the *venules at the basilar membrane* (VLBM) (Figs 7, 9) They have been demonstrated in all mammals investigated so far The CVL basal to the venules at the basilar membrane appear more numerous and of a more delicate caliber than the corresponding vessels in the other mammals examined There are occasional *anastomoses* and loop formations between nearby CVL The CVL in the scala tympani merge to larger collecting venules and veins which finally empty into the spiral modiolar vein The CVL drain all the spiral capillary structures (i e, stria vascularis, the vessel of the

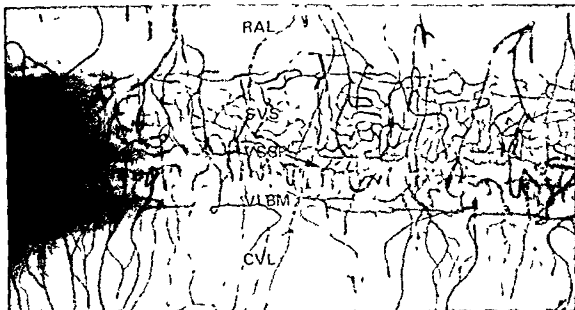


Fig 7 Ch... wall ba... d stribut... laris (*S..*) is a broad vascular net formed by small capillaries The vessel of the spiral prominence (*VSSP*) is formed by many short spirally cours ng segments of capillaries in the spiral prominence All vessels of the external wall are drained by collecting venules (*CVL*) which make an omega formed turn around the attach ment of the basilar membrane and then run spirally forming the venules at the basilar membrane (*VLBM*) before turning basally



spiral prominence and the arteriovenous anas tomoses) There are no apparent perivascular spaces seen surrounding the vascular wall of the collecting venules

Apex

Apical parts of the cochlea are characterized by a simplification of the vascular pattern However, this is much less apparent in the chinchilla cochlea than in other mammals in vestigated In the external wall all described vascular structures are easily demonstrated very close to the apical end (Fig 10) Likewise, in the spiral lamina, the vascular structures are regularly found up to the apical end of the cochlea (Fig 11) A regular finding which has not been demonstrated in other mammals is the occurrence of numerous spirally coursing, very delicate branches of the RAL and CVL

Fig 8 Ch nchilla cochlea Transverse section Basal turn The radiating arterioles have a pronounced serpentine course centrally in the scala vestibuli fr ramification the separate branches lie close



central in the spiral lamina and around the helicotrema

Basal end

The vascular pattern of the basal end of the chinchilla cochlea is very similar to that in the other mammals investigated In the external wall, the radiating arterioles and the collecting venules take a more oblique course between the oval and round windows The collecting venules empty in a well formed *vein of the round window* (VRW) which turns around the apical aspect of the round window and finally empties into the vein of the cochlear aqueduct The *vein of the cochlear aqueduct* (VCAQ) is formed where the spiral modiolar vein, the vein of the round

Fig 9 Chinchilla cochlea Apico-basal section External wall second turn There are no connections between the capillaries of the stria vascularis (*SVS*) and the spirally coursing vessel of the spiral prominence (*VSSP*). Both are supplied and drained by separate radiating arterioles and collecting venules which form a spirally running arcade the venules at the basilar membrane (*VLBM*) immediately basal to the attachment of the basilar membrane

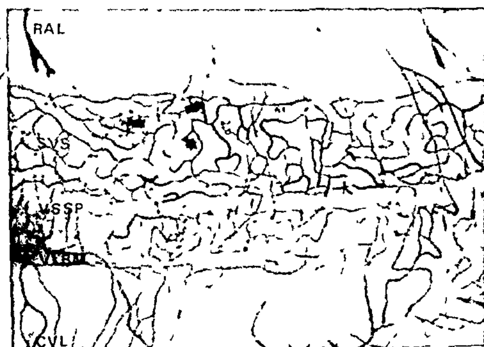


Fig 10 Chinchilla cochlea Apico-basal section Third turn. The apical simplification of the vasculature of the external wall is much less pronounced in the chinchilla than in other mammals The stria vascularis (SVS) still forms a broad vascular net All types of vessel are easily identified radiating arterioles (*RAL*) the vessel of the spiral prominence (*VSSP*) the venules at the basilar membrane (*VLBM*) collecting venules (*CVL*).

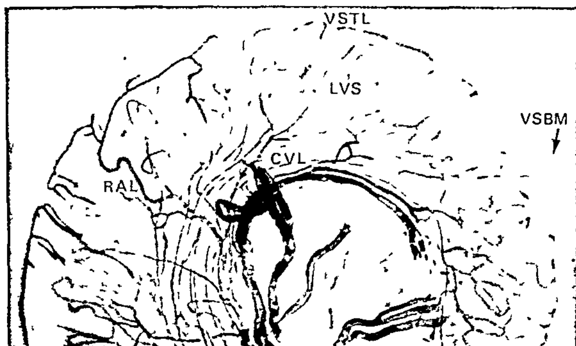


Fig 11 Chinchilla cochlea. Transverse section Spiral lamina, third turn The apical simplification of the vasculature is not so pronounced as in other mammals All vascular structures including the occasionally vessels

window, and the vestibulo-cochlear vein merge (Fig 12) The vein of the cochlear aqueduct makes a more abrupt turn at the basal end than in the other mammals investigated At the basal end, the common cochlear artery gives off the vestibulo-cochlear artery which mainly supplies the vestibular part of the labyrinth However, on its course to the vestibulum, the vestibulo-cochlear artery supplies the extreme basal end of the spiral lamina and the modiolus wall (Fig 13) All vascular structures of the external wall are demonstrated to the extreme basal end The stria vascularis is well formed between the windows, contrary to the human and rabbit cochlea As in other mammals, the scala vestibuli at the extreme basal end is supplied by radiating arterioles deriving from the vestibulo-cochlear artery and coming around the "hook" region and turning apically (Fig 14) At the level of the oval window, they anastomose with the radiating arterioles coming from the apical side The sparse capillary net formed here in the scala vestibuli is drained by a large collecting venule protruding from the scala tympani to the scala vestibuli (Fig 14)

DISCUSSION

The vasculature of the cochlea has so far been examined with the present technique in man, Rhesus monkey, rabbit, guinea pig, and chinchilla (Axelsson, 1968, 1971, 1972, 1974, Axelsson & Lind, 1973) In none of these mammals was a regular, good, and even Prussian blue injection achieved as easily as in the chinchilla The chinchilla cochlea is characterized by a pronounced richness of vessels which give the visual impression of more abundance than in the other mammals Like these, the vasculature is segmentally arranged with radiating arterioles and collecting venules supplying and draining a fairly small segment of the cochlea both in the external wall and in the spiral lamina The more specific characteristics of the chinchilla ... summary are

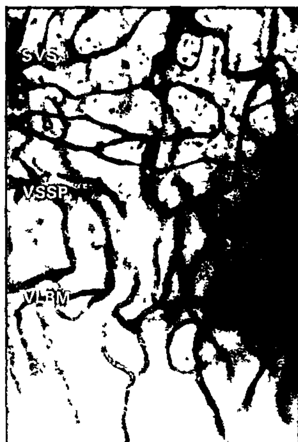


Fig 9 Chinchilla cochlea Apico-basal section External wall second turn There are no connections between the capillaries of the stria vascularis (*SVS*) and the spirally coursing vessel of the spiral prominence (*VSSP*) Both are supplied and drained by separate, radiating arterioles and collecting venules which form a spirally running arcade the venules at the basilar membrane (*VLBM*) immediately basal to the attachment of the basilar membrane

central in the spiral lamina and around the helicotrema

Basal end

The vascular pattern of the basal end of the chinchilla cochlea is very similar to that in the other mammals investigated In the external wall, the radiating arterioles and the collecting venules take a more oblique course between the oval and round windows The collecting venules empty in a well formed *vein of the round window* (VRW) which turns around the apical aspect of the round window and finally empties into the vein of the cochlear aqueduct The *vein of the cochlear aqueduct* (VCAQ) is formed where the spiral modiolar vein, the vein of the round

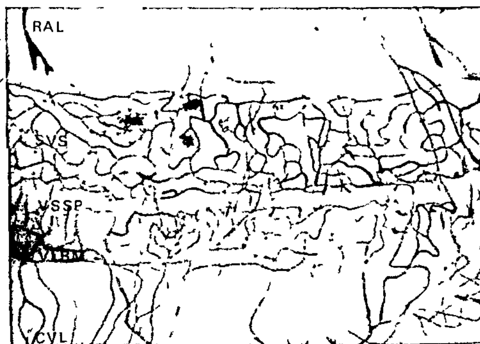


Fig 10 Chinchilla cochlea Apico basal section Third turn The apical simplification of the vasculature of the external wall is much less pronounced in the chinchilla than in other mammals The stria vascularis (SVS) still forms a broad vascular net All types of vessel are easily identified radiating arterioles (*RAL*) the vessel of the spiral prominence (*VSSP*) the venules at the basilar membrane (*VLBM*) collecting venules (*CVL*)

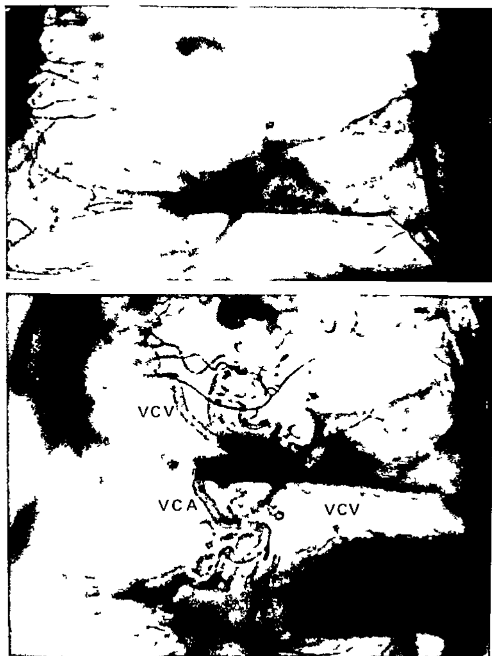


Fig 13 Chinchilla cochlea Apico-basal section Basal end *Above* at the extreme basal end of the spiral lamina (*left*) the ductus reuniens is shown in relation to the vestibulo-cochlear artery (*VCA*) and the vestibulo-cochlear vein (*VCV*) (*below*)

(Hawkins et al 1968 Hilding 1969 Johnsson 1972 Johnsson & Hawkins 1972) Consequently the degree to which this vessel contributes to the oxygen supply of the adult organ of Corti can be questioned

The greatest difference from the other mammals examined was the vascular pattern of the scala vestibuli with very sparse occurrence of the otherwise regularly demonstrated capillary vessels above the attachment of the vestibular

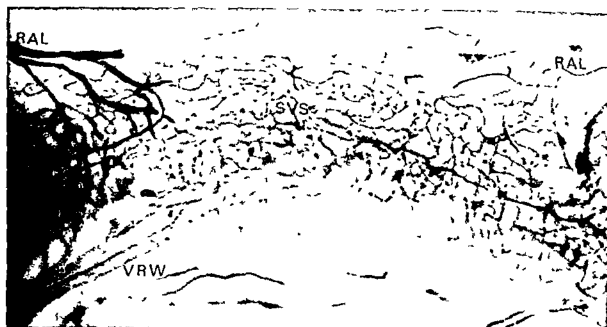


Fig 14 Chinchilla cochlea Longitudinal section Basal end In the external wall between the oval window (*above*) and the round window (*below*), radiating arterioles (*RAL*) from the cochlea (*left*) take an oblique course Radiating arterioles (*RAL*) coming from the vestibulum turn in a retrograde direction upward around the extreme basal end (*right*) A sparse capillary net is formed where these radiating arterioles merge The stria vascularis (*SVS*) is well formed to the extreme basal end The collecting venules are short, and empty into the vein of the round window (*VRW*)

membrane In the other mammals, the vessel at the vestibular membrane constitutes the basal ...inal border of a capillary net which could ... be demonstrated in the chinchilla cochlea ... vessels are supposedly of great importance for the formation of perilymph However, the perivascular spaces around the radiating arterioles in these regions are well formed, which fact may indicate participation in the fluid balance

The ease with which the chinchilla is conditioned for hearing testing, the favorable accessibility of the cochlea and middle ear for electrophysiological measurements, and the regularly good results of the contrast injection make the chinchilla an ideal animal for research of auditory functions In a future report (Axelsson et al, 1974*b*), audiological and histopathological findings in the noise-exposed chinchilla will be reported

ZUSAMMENFASSUNG

Die Gefässanatomie der Chinchilla-cochlea wurde mit Berliner Blau Injektionen dargestellt Im allgemeinen ahnelt das Gefassmuster dem anderer Sauger Überall findet man einen Reichtum an Gefassen, die aber von feinerer Struktur zu sein scheinen Besondere Befunde der Chinchilla-cochlea waren die Venen der Scala tympani, die aus Sammelvenolen gebildet werden und parallell zu der spirallaufigen Vene im Modiolus gehen Weiter konnte kein regelmässiges Vorhandensein eines Gefasses unter dem Cortischen Tunnel gezeigt werden Dieses Gefass ist vermutlich von grosser Bedeutung fur den Sauerstoffaustausch im Cortischen Organ Die Aussenwand der Scala vestibuli wird im Unterschied zu anderen Saugern ausschliesslich von arteriellen Gefassen versorgt Die Stria vascularis ist breit und gut entwickelt, und zwar auch an der Cochleaspitze und am basalen Ende

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CARDIOVASCULAR RISK FACTORS AND HEARING LOSS

A Study of 1000 Fifty-Year-Old Men

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(Received May 16, 1974)

Abstract The hypothesis that cardiovascular risk factors might be of importance in the development of sensorineural hearing loss was tested in a material of 1000 fifty-year-old men No significant correlations were found. The present study confirmed the well known observation that the left ear usually is poorer than the right Hearing loss in the right ear was found to be related to the smoking habits in the groups with no history of noise exposure The explanation for this is discussed Hearing loss was more common in social class 3 than in the other social classes This difference was principally referable to noise exposure but also to conductive hearing loss A prospective study of this material will further analyze the question concerning a possible relationship between ovascular risk factors and hearing loss

The deterioration of hearing related to aging is a well known phenomenon It is predominantly a matter of progressive sensorineural high tone loss, *which has been observed to start already* during the third decade of life Most of the available information on morphological and functional changes in presbycusis has been reviewed by Schmidt (1967) In summing up he points out "we all carry to our senium the cumulation of harms done to our hearing acuity in a lifetime"

Among factors discussed are principally noise exposure and vascular disease

The importance of cardiovascular disease is unclear No relation between hearing loss and cardiovascular disease was noted by Bunch et al (1929, 1931) or Miller & Ort (1966) On the other hand a relationship between a certain morphological type of presbycusis and vascular disease has been suggested by Schuknecht (1964) Weston (1964) reported that the age of onset and the progress of presbycusis were related to circulatory disturbances, among other factors

Audiological studies performed in different areas of the world have indicated a possible relationship between hearing loss and high intake of saturated fats, high cholesterol levels, atherosclerosis and coronary heart disease (Rosen et al 1962, 1964*a*, 1964*b*, 1965, 1970*a*, 1970*b*) Great attention has focused on Rosen's reports according to which a change in the fat composition of the diet in the direction from saturated to unsaturated fat was followed not only by a reduction of the incidence of coronary heart disease but also of a diminished impairment of hearing *(Rosen et al, 1970a)*

An observation which is also of interest in this connection is the study by Ismail et al (1973) showing that physical exercise which resulted in improvement in different cardiovascular parameters, did not affect the hearing thresholds, but resulted in an improved ability to recover from a temporary threshold shift induced by noise

Against this background it was judged to be of interest to perform tone audiometry in connection with an extensive health examination survey of a large unselected material of 50-year-old men The health examination was intended to identify risk factors for cardiovascular disease, such as hypertension, elevated blood lipid levels and smoking

Table I *Factors used for correlation studies with hearing loss*

1	Systolic blood pressure
2	Diastolic blood pressure
3	Heart rate
4	Serum-cholesterol
5	Serum triglycerides
6	Uric acid
7	Hematocrit
8	Glucose tolerance
9	Smoking habit
10	Noise exposure

The purpose of the present investigation was to search for possible correlations between hearing loss and cardiovascular risk factors, separate as well as in combinations Studies concerning smoking habits and hearing function do not seem to have been reported earlier

MATERIAL

The material consisted of 1 000 men, aged fifty, who were consecutively selected from a health examination survey, with the special aim of identifying risk factors for cardiovascular disease in healthy middle-aged men in the City of Uppsala The examination was performed in the morning between 7 15 and 9 00 a m The participants were asked to come after an over night fast and not to have smoked after midnight The researchers were the same during the whole examination period The participation rate was 83 9 % Table I shows the "risk factors" which have been used to study the interrelationship with the amount of sensorineural hearing loss

Grouping of the material according to social classes was based on interview report on occu pation The following three classes were used (Andersen et al, 1970)

Social class 1 Professionals and academically trained persons, high officials, proprietors and managers of large businesses and industry

Social class 2 Clerical and sales workers, foremen self-employed craftsmen, small business proprietors, elementary school teachers

Social class 3 Laborers, service workers, operatives, salaried craftsmen

METHODS

The *heart rate* was counted and the *blood pressure* measured after 10 minutes of rest in the lying position

The *serum cholesterol and the triglycerides* were assayed in an Isopropanol extract of serum by using a Technicon dual channel system (N-24 A and N-70) The *hematocrit* was measured with a micromethod with capillary tubes *Uric acid* was determined by a wolframat method

The *intravenous glycose tolerance* test (IVGTT) was performed with a glycose dose of 0 5 g per kg bodyweight administered as a 50 % solution Blood samples for determination of glycose in plasma were taken at 10 min intervals over 1 hour for estimation of glycose tolerance, which was expressed as a *K*-value calculated from the formula $K = \ln 2 \times 100/T_{1/2}$ where the $T_{1/2}$ is the time in minutes required for the concentration to be reduced by half its value This IVGTT was performed in a random sample of 594 men in this study

The *hearing* was tested with a pure tone audiometer at the frequencies 500, 1 000, 2 000, 3 000, 4 000 and 6 000 Hz bilaterally With the sound insulated headphones used the investigation room was found to be silent enough to permit relevant threshold determinations down to 15 dB (ISO, R 389), which was regarded as non-significant hearing loss and lower values were accordingly not registered

The individuals were interviewed by questionnaire concerning history of *ear disease, noise exposure* and *smoking habits*

CLASSIFICATION OF THE MATERIAL AND STATISTICAL PROCEDURES

A computer IBM 370/155 was used for sorting and calculations A check was included that all parameters fell within reasonable limits Correlation studies were performed between hearing loss and the factors in Table I Moreover smoking *habits, history of noise exposure and of ear disease* were included in the statistical calculations

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Against this background it was judged to be of interest to perform tone audiometry in con nection with an extensive health examination survey of a large unselected material of 50-year old men The health examination was intended to identify risk factors for cardiovascular dis ease, such as hypertension, elevated blood lipid levels and smoking

Table II *Distribution (%) of hearing loss in social classes in 1 000 males, aged 50 The amount of hearing loss is expressed as the sum of dB hearing loss of both ears at five frequencies 1 000–6 000 Hz With the screening level of 15 dB an amount of hearing loss of 150 dB thus means no significant hearing loss*

Social class	N	Amount of hearing loss in dB 150–180 %	185–225 %	230–295 %	300–385 %	390– %	
1	130	23.8	24.6	17.7	21.5	12.3	100
2	402	25.4	23.9	20.4	15.7	14.7	100
3	468	16.0	13.9	21.6	22.2	26.3	100

With the screening level of 15 dB, a sum of hearing loss of 150 dB means "no significant hearing loss", whereas a sum of 390 dB or more may be regarded as severe hearing loss As seen in table II, hearing loss was most common as well as most pronounced in social class 3, in which 26.3% had severe hearing loss while only 16.0% were free from significant hearing loss The corresponding figures in social class 1 were 12.3% and 23.8% respectively This difference in distribution of hearing loss between social classes 1 and 3, seen in the table, was found to be statistically significant

It seems that conductive hearing loss was also more prevalent in social class 3 than in social class 1 Among the 238 subjects, who had possible conductive loss according to the criteriea given previously 53.4% belonged to social class 3 and only 11.3% to social class 1 The corresponding figures among the remaining 762 subjects were 44.6% belonging to social class 3 and 13.5% to social class 1 This difference between the two social classes was however not statistically significant There was also a greater occurrence of men with a history of ear disease in social class 3 than in the other two classes

Noise exposure

In the total material a history of noise exposure was statistically more prevalent in social class 3 than in any of the other two classes

In material B there were 388 individuals with a history of civil and/or military noise exposure, whereas the remaining 374 cases denied such exposure The hearing loss was found to be significantly greater in the noise-exposed group, the difference being most pronounced around 4 000 Hz ($p<0.001$) These observations were valid for the left as well as for the right ear

In cases with asymmetrical hearing data (difference ≥ 10 dB) the hearing loss was in most cases greater in the left ear than in the right ear The number of cases with left-sided inferiority was found to increase towards higher frequencies to be most pronounced at 4 000 Hz In this respect there was no difference between those who had answered "yes" or "no" to the question concerning noise exposure

Smoking habits

In the total material there were 509 smokers and 276 who had never smoked Neither material A nor material B showed any significant differences in hearing loss related to smoking habits per se However, smoking and hearing loss may still have an etiological common denominator, according to the following

Combinations of noise exposure and smoking habits

The four sub-groups in material B (B1–B4) listed above were selected for correlation studies between, on one hand, hearing loss and, on the other, history of smoking habits and noise exposure Hearing data in the four groups are presented in Table III

Only one indication of a possible effect of smoking was found Among the 92 individuals (B4) who had smoked more than 10 cigarettes daily but had not been exposed to noise, the amount of right-sided hearing loss was significantly greater ($p<0.001$) than in the 105 individuals (B2) who had never smoked and had not been exposed to noise (Figs 1 and 2) Among these smokers, who were not exposed to noise, there was no right-sided superiority in

Table III *Hearing loss related to history of noise exposure and smoking habits in 377 males, aged 50*

The hearing loss is given as median values in dB for the right and the left ears respectively at 2 000–6 000 Hz

		No history of noise exposure		History of noise exposure	
Hz	Ear	Non smokers $N=105$	Smokers $N=92$	Non smokers $N=101$	Smokers $N=79$
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3 000	Right	19 2	25 7	22 8	24 7
3 000	Left	22.5	23 2	29 8	28 0
4 000	Right	23 0	31 0	34 0	34 5
4 000	Left	28 1	29 5	39 1	40 6
6 000	Right	26 3	28 3	35 8	29 6
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hearing but instead an insignificant tendency towards left sided superiority These findings refer to 3 000 and 4 000 Hz

No similar difference was found in the noise exposed material between the 79 individuals (B3) who had smoked 10 cigarettes daily and the 101 individuals (B1) who had never smoked

DISCUSSION

phenomenon that the left ear usually is ›orer than the right ear, has been observed

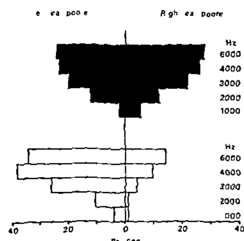


Fig 1 The distribution of a asymmetrical hearing loss at five cardinal frequencies among 197 males aged 50 with no history of noise exposure The upper part of the figure shows the results from group B4 (heavy smokers) and the lower part from group B2 (never smoked) Asymmetry means a difference of ≥10 dB

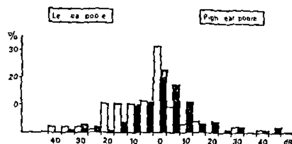


Fig 2 The distribution of differences in hearing loss between right and left ears at 4 000 Hz in 197 males aged 50 with no history of noise exposure White bars represent the 105 non smokers (group B2) black bars the 92 smokers (group B4)

previously (Glorig & Roberts, 1965) Asymmetrical hearing impairment of this kind has been ascribed to noise, especially from fire arms In our study leftsided inferiority was found not only among the individuals with noise exposure but also in those who denied noise exposure It must be kept in mind however, that most of the subjects who denied noise exposure had nevertheless had a period of military service in their past The leftsided inferiority, statistically observed, might also be of a more genuine origin since the phenomenon has been observed among male children as well (Kannan & Lipscomb 1974)

Another observation partly connected to noise exposure was that hearing impairment was more common in social class 3 than in the other classes This difference was found to be due not only to noise exposure but also to greater occurrence of conductive hearing loss

A correlation between hearing impairment and cardiovascular risk factors has been discussed, not at least considering the publications of Rosen et al (1965, 1970) They reported that a long term change of the fat diet from saturated to poly unsaturated fat intake was followed by a decrease not only in the incidence of coronary heart disease but also in the deterioration in hearing In our study we did not find any correlation between hearing loss and cardiovascular risk factors or combinations of risk factors

The only exception was the peculiar finding that the hearing in the right ears was poorer in heavy smokers not exposed to noise than in the individuals who were not exposed to noise and who had never smoked This difference was statistically significant Among the individuals with a history of noise exposure no similar difference was found between the heavy smokers and those who had never smoked We cannot find any obvious explanation for this observation It may be possible that smoking, or associated conditions in heavy smokers, can result in a deterioration of hearing, with an effect only discernible in the absence of noise trauma Why such a hypothetical effect should involve predominantly the right ear, which otherwise usually is superior, seems obscure It should be stressed that it is not necessarily a matter of causality

The question of a possible relationship between cardiovascular risk factors and hearing loss motivates further investigation, especially of a prospective character The follow-up of our material will offer such a possibility

ZUSAMMENFASSUNG

Die Hypothese dass kardiovaskulare Risikofaktoren fur die Entwicklung der Schwerhorigkeiten von Bedeutung sein konnten, wurde an 1000 50-jahrigen Mannern getestet Keine signifikanten Korrelationen wurden festgestellt Die vorliegende Studie bestätigt die wohlbekannten Beobachtungen, dass das linke Ohr gewohnlich mehr geschadigt ist als das rechte was wahrscheinlich durch Larmeinwirkung hervorgerufen wird In den Gruppen mit keiner Larmeinwirkung in der Anamnese wurde je doch eine Korrelation zwischen Horstorungen des rechten Ohres und den Rauchgewohnheiten gefunden Die Er klärung dafur wird diskutiert Horstörungen traten in der Sozialgruppe 3 haufiger auf als in den anderen Gruppen Dieser Unterschied war hauptsächlich auf Larmeinwirk ung zuruckzufuhren, aber auch auf Schalleitungsstorung Eine prognostische Bearbeitung dieses Materials wird ferner das Problem eines moglichen Zusammenhangs zwischen kardiovaskularen Risikofaktoren und Gehor storungen veranschaulichen konnen

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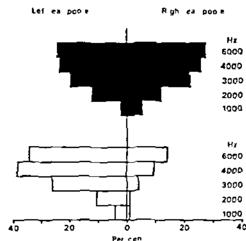


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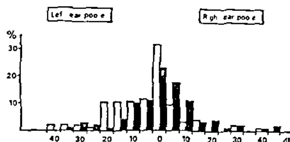


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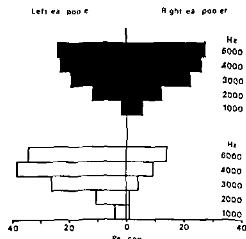


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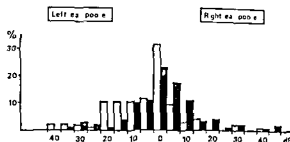


Fig 2 The distribution of differences in hearing loss between right and left ears at 4 000 Hz in 197 males aged 50, with no *history of noise exposure White bars re*present the 105 non smokers (group B2) black bars, the 92 smokers (group B4)

Another observation, partly connected to noise exposure was that hearing impairment was more common in social class 3 than in the other classes This difference was found to be due not only to noise exposure but also to greater occurrence of conductive hearing loss

A correlation between hearing impairment and *cardiovascular risk factors* has been discussed, not at least considering the publications of Rosen et al (1965, 1970) They reported that a long-term change of the fat diet from saturated to poly unsaturated fat intake was followed by a decrease not only in the incidence of coronary heart disease but also in the deterioration in hearing In our study we did not find any correlation between hearing loss and cardio vascular risk factors or combinations of risk factors

physikalischen Faktoren, wie die Körpertemperatur des Versuchstiers, die Temperatur und Zusammensetzung des Perfusionsmittels, die Länge der Narkose und weitere, mußten in ihrer Wirkung konstant gehalten werden, um zu eindeutigen Ergebnissen zu gelangen

METHODIK

Die 200 bis 300 g schweren Meerschweinchen wurden durch intraperitoneale Gabe von 1,5 ml 10% Äthylurethan pro 100 g Körpergewicht narkotisiert Die Haut wurde retroaurikulär sowie entlang der Mandibula bis 5 mm vor dem Mundwinkel durchtrennt, die Arteria cervicalis subcutanea und die Vena jugularis externa wurden unterbunden Nun konnte nach Fraktur in der Mitte des horizontalen Astes der Mandibula und Luxation des lateralen Unterkieferteils der Proc styloides durchtrennt und mit seiner Muskulatur entfernt werden Nach Darstellung der Bulla wurde diese mit der Lüerschen Zange vorsichtig von ventral eröffnet und der Knochen bis zum Rand des Os tympanicum abgetragen Auch die angrenzenden kaudalen und dorsalen Bullateile wurden entfernt

Durch Registrierung der RMP zwischen 20 Hz und 20 kHz (L_{Step} = 75 dB bei 1 kHz) wurde die uneingeschrankte Funktionsfähigkeit des peripheren Hörorgans nach beendeter Präparation kontrolliert Der Einfluß der Bohrlochlokalisation konnte durch Bohrungen mit einem Durchmesser von 1 mm in 0,5, 1 und 2,5 mm Entfernung vom runden Fenster (d h. Abstand zwischen den Rändern des runden Fensters und des Bohrlochs) bei je 10 Ohren in der Scala tympani nachgewiesen werden

Wir untersuchten weiter die Wirkung von Druckveranderungen zwischen −30 cm und +30 cm Wassersäule, die durch eine mit Gewebekleber (Histoacryl-*N* Blau) in die Scala tympani eingeklebte Glaskapillare auf das Innenohr einwirkten Wie bei der vorhergehenden Versuchsreihe diente auch hier die Messung des RMP als Bewertungsmaßstab

Veränderungen der Stromungsverhaltnisse als Folge von Druckänderungen ließen sich durch Zusatz von 2,5 mg% Patentblau, einem Triphenylmethanfarbstoff, zum Perfusionsmittel schon durch Inspektion der Schnecke, wie auch durch Punktion einzelner Kochleawindungen und der Cisterna cerebellomedullaris mit nachfolgender Untersuchung der entnommenen Flüssigkeit auf Blaufärbung feststellen

Die Perfusionsgeschwindigkeit wurde durch *eine Peristaltic miniflow pump Typ 304 der* Firma Unipan Warszawa bzw eine Dauerinfusionspumpe der Firma H Diel Leipzig zwischen 1 und 50 µl/min variiert und der Einfluß auf die Funktion des Innenohres durch RMP Messungen bestimmt.

ERGEBNISSE

Bei der Präparation bereitet die Entfernung des kaudalen Bullaabschnitts besondere Schwierigkeiten, da das kranial angrenzende Os tympanicum und das Trommelfell unverletzt bleiben müssen, trotzdem sie nach der erforderlichen breiten Freilegung der Kochlea nur noch durch eine schmale Knochenspange kranial am Schädel fixiert sind Etwa 1 bis 2 mm Sicht- und Arbeitsraum bleiben an dieser Stelle bis zu einem kaudal angrenzenden Venenplexus, bei dessen Läsion die folgende starke Blutung meist zu einem Abbruch des Versuchs zwingt

Durch eine sorgfaltige Abdeckung der Bullarander mit Zellstoff bzw Gelaspon kann das Einfließen von Wundsekret und Blut in das Mittelohr verhindert werden, das auf dem Trommelfell, der Gehörknöchelchenkette oder den Fenstern lagernd stets zu einer Minderung der RMP durch eine Störung der Schallübertragung führt Wenn Blut eingeflossen ist, gelingt es nur in Ausnahmefallen, dieses wieder zu entfernen, ohne Schäden im Mittelohr zu verursachen

Beim Bohren ist die Drehrichtung der Diamantfräse stets so zu wählen, daß die Knochenteilchen aus dem Mittelohr herausgeschleudert werden, da sonst die Schalleitung ebenfalls gestört wird Dem Bohrloch von 1 mm Durchmesser muß auch die Größe der konisch enden-

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DIE WIRKUNG METHODISCHER FAKTOREN AUF DAS MIKROPHONPOTENTIAL DER MEERSCHWEINCHENKOCHLEA BEI DER PERILYMPHATISCHEN PERFUSION

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(Eingegangen am 19 Februar, 1974)

Abstrakt Nur bei der Anlage der Bohrlocher in unmittelbarer Nahe des runden Fensters ist die RMP Minderung vernachlassigbar klein wahrend sie mit zunehmender Entfernung von diesem zunimmt Erst bei Druckwerten von uber ±30 cm Wassersaule kommt es wahrscheinlich durch Verletzung des Ringbandes bzw der runden Fenstermembran in seltenen Fallen schon eher, zu einem starken Abfall der RMP Dabei fließt bei einem Perfusionsdruck uber +5 6 cm Wassersaule Perfusionsflussigkeit in den Liquorraum Bei einem niedrigeren Druck wird die Perfusionsflussigkeit durch Liquor verdunnt Die Perfusionsgeschwindigkeit von *1 bis 50 µl/min hat keinen Einfluß auf die Funktion des* Cortischen Organs

die Stoffwechsellage des Innenohrs, speziell des Cortischen Organs nach Schallbelastung studieren und spater beeinflussen zu können, wandten wir uns der Perfusion der perilymphatischen Raume zu Diese Methode gestattet es, interessierende Wirkstoffe moglichst nahe an den Schadigungsort heranzubringen, verschiedene Fragen der Physiologie des Innenohrs (Butler et al, 1962, Thalmann & Bornschein, 1964, Honrubia et al, 1964, Silverstein, 1970 u a) und die Wirkung verschiedener Stoffe auf das intakte sowie geschadigte Cortische Organ zu klaren (Davis et al, 1955, Cutt, 1963, Kuipers et al, 1967 Prazma, 1969, Martinez, 1969, Okomura, 1970, Chou & Vosteen, 1971, u a)

Da als Ausgangspunkt der Untersuchungen unser Lärmschädigungsmodell (*Wagner et al* im Druck) dienen sollte, war neben einem intakten Innenohr ein funktionstuchtiger, moglichst unversehrter Schalleitungsapparat Voraussetzung fur die Versuche, ebenso die Moglichkeit der Perfusion bei gleichzeitiger Beschallung durch ein geschlossenes System und der Ableitung von Mikrophonpotentialen vom Rand des runden Fensters

Viele Faktoren beeinflussen während der präparativen Vorbereitung und Durchfuhrung die *Funktion* des peripheren *Hörorgans Diese* Störungen mussen möglichst ausgeschaltet, wenigstens aber erkannt und in der Auswertung korrigiert werden

Butler & Honrubia (1963) untersuchten den Einfluß des hydrostatischen Drucks auf die Kochleapotentiale, Sohmer & Feinmesser (1967) die Wirkung des unterschiedlichen osmotischen Drucks, Tsunoo & Perlman (1969) die Abhängigkeit der kochleären Mikrophonpotentiale vom pH des *Perfusionsmittels und Jung (1971 und* 1974) die Verhaltnisse bei unterschiedlichem pH und hydrostatischem Druck

Wir pruften den Einfluß der Lage der Bohrlocher, des Perfusionsdruckes und der Perfusionsgeschwindigkeit auf die vom runden Fenster abgeleiteten Mikrophonpotentiale (RMP) wie auch die Stromungsverhaltnisse im Perilymphraum und Aquaeductus cochleae bei erhohtem Druck

Alle anderen, die Funktion des peripheren Hororgans beeinflussenden biochemischen und

physikalischen Faktoren, wie die Korpertemperatur des Versuchstiers, die Temperatur und Zusammensetzung des Perfusionsmittels, die Lange der Narkose und weitere, mußten in Ihrer Wirkung konstant gehalten werden, um zu eindeutigen Ergebnissen zu gelangen

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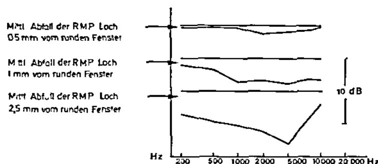


Abb 1 Mittlere Minderung der RMP bei unterschiedlicher Lokalisation der Bohrlöcher Detailangaben siehe Tab I

den Glaskapillare angepaßt sein Eine zu geringe Dicke kann zu zu tiefem Eindringen in die Kochlea und dadurch zu Verletzungen der Innenohrstrukturen führen Zu dicke Kapillaren lassen sich nicht genügend weit einführen und werden dann durch den Gewebekleber verschlossen, der natürlich auch nicht auf Teile des Schalltransformationssystems gelangen darf

Die Änderung des RMP in Abhängigkeit von der Lokalisation der Bohrlöcher sind in Abb 1 und Tabelle I dargestellt Nur bei Anlegen des Bohrlochs unmittelbar am runden Fenster fand sich ein RMP-Abfall, der vernachlässigbar klein ist (0,6 dB mittlere Minderung) Die mittlere Differenz zum Ausgangsfrequenzgang (Mittelwertbildung aus den mittleren RMP-Minderungen bei 200, 500, 1 000, 2 000, 4 000, 7 000 und 10 000 Hz) betrug bei 1 mm Entfernung 2,8 dB, bei 2,5 mm 4,7 dB, wobei der Abfall der RMP frequenzabhängig unterschiedlich war

Der Abfall der Potentiale wird sicher nicht durch Verletzungen der Innenohrstrukturen hervorgerufen, da nach einem Verschluß der Bohrlöcher die Höhe des Ausgangsfrequenzganges wieder erreicht wird Ein elektrischer Nebenschluß als Ursache konnte durch folgenden Versuch ausgeschlossen werden Beim Verschluß der Bohrlöcher wurde ein isolierter Silberdraht so eingeklebt, daß ein leitendes Ende in der Perilymphe lag, das zweite mit der unmittelbaren Umgebung des Bohrloches bzw mit der Mittelohrschleimhaut Kontakt hatte Dabei blieben die Potentiale unverändert hoch Erst wenn die Schnecke wieder eröffnet wurde, sanken sie auf ihren vorherigen Wert ab

Druckänderungen im perilymphatischen Raum zwischen −30 cm und +30 cm Wassersäule wirkten sich bei unseren Untersuchungen nicht nachteilig auf die Höhe der RMP aus

Wir bestimmten nun den Druck, bei dem die mit Patentblau angefärbte Perfusionsflüssigkeit

Tabelle I *Mittlere RMP-Minderungen bei Lokalisation der Bohrlöcher in 0,5, 1,0 und 2,5 mm Entfernung vom runden Fenster*

	Frequenz in Hz							$\frac{\sum_{i=1}^{7} \bar{x}_i}{7}$
	200	500	1 000	2 000	4 000	7 000	10 000	
Loch 0,5 mm vom runden Fenster (n 10)								
Mittl RMP-diff in dB	$\bar{x}_1 = 0,3$ $s = 0,46$	$\bar{x}_2$ 0,2 s 0,40	$\bar{x}_3$ 0,3 s 0,65	$\bar{x}_4 = 1,2$ $s = 1,60$	$\bar{x}_5 = 1,0$ $s = 1,14$	$\bar{x}_6 = 0,7$ $s = 0,79$	$\bar{x}_7 = 0,2$ $s = 0,60$	0,6 $s = 0,41$
Loch 1 mm vom runden Fenster (n 10)								
Mittl RMP-diff in dB	$\bar{x}_1 = 1,0$ s 1,26	$\bar{x}_2$ 1,7 s 1,63	$\bar{x}_3 = 3,5$ $s = 1,05$	$\bar{x}_4 = 3,2$ s 1,33	$\bar{x}_5 = 3,8$ $s = 1,17$	$\bar{x}_6 = 3,2$ $s = 1,47$	$\bar{x}_7 = 3,3$ $s = 1,75$	2,8 $s = 1,04$
Loch 2,5 mm vom runden Fenster (n 10)								
Mittl RMP-diff in dB	$\bar{x}_1 = 3,6$ $s = 1,27$	$\bar{x}_2$ 4,7 s 1,80	$\bar{x}_3$ 5,3 $s = 0,95$	$\bar{x}_4 = 6,1$ $s = 2,54$	$\bar{x}_5 = 8,0$ $s = 3,70$	$\bar{x}_6 = 4,3$ $s = 2,81$	$\bar{x}_7 = 1,0$ $s = 3,03$	4,7 $s = 2,17$

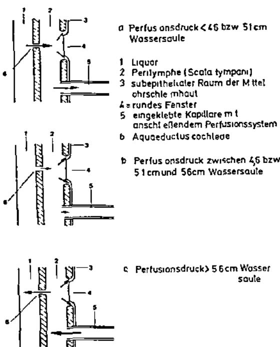


Abb 2 Stromungsverhaltnisse im Aquaeductus cochleae und der Scala tympani bei unterschiedlichem Perfusionsdruck → = Richtung und Stärke des Flussigkeitsstroms

bei erhaltenem RMP in die Kochlea hineinzufließen begann Er lag bei +5,6 cm Wassersäule ±0,87 cm Unterhalb eines 5–10 mm tieferen und deshalb mit unserer relativ trägen Meßmethodik nicht genauer zu bestimmenden Druckes stieg Perilymphe in die Glaskapillare Der zwischen diesen beiden Werten liegende schmale Bereich war durch einen Stillstand oder ein kaum merkliches Einfließen des Perfusionsmittels gekennzeichnet Sobald 5,6 cm Wassersaule uberschritten wurden, stellten wir eine Blaufärbung der Perilymphe und des Liquors in den ersten 3 mm der Scala tympani vom runden Fenster an fest Bei einer Steigerung des Druckwertes auf das Doppelte anderten sich diese Verhältnisse nicht, die Einströmgeschwindigkeit wurde nur großer (siehe Abb 2*a*)

Perfusionsgeschwindigkeiten von 1 bis 50 µl/min hatten keinen Einfluß auf die Größe der Mikrophonpotentiale, solange der Druck nicht uber +30 cm Wassersäule anstieg

DISKUSSION

Bei der notwendigen breiten Freilegung des Mittelohrs durch die oben beschriebene Technik waren Schalleitungsstorungen durch Serum, Blut- oder Knochensplitterauflagerung, Verletzung des Trommelfells, des Os tympanicum, der Gehorknochelchenkette und der Fenster vermeidbare Fehlerquellen Nach deren Ausschaltung entsprach der Mikrophonpotentialfrequenzgang dem des unverletzten Tieres

Jung (1974) stellte fest, daß die MP in der Regel nach Anbohren der Kochlea deutlich absinken Bei unseren Untersuchungen zeigte sich demgegenuber eine Abhängigkeit von der Lokalisation der Bohrlocher in der Scala tympani In unmittelbarer Nähe des runden Fensters kann der RMP Abfall sogar vernachlässigt werden Die mittlere RMP-Minderung beträgt hier 0,6 dB bei $s = 0,41$ dB und weicht damit statistisch nicht von der Stichprobe der Ausgangsfrequenzgänge ab Mit zunehmender Entfernung vom runden Fenster wird sie mit 2,8 dB ($s = 1,04$) bei 1 mm und 4,7 dB ($s = 2,17$) bei 2,5 mm größer Die RMP-Differenz zwischen 0,6 und 2,8 ist bei einem t-Wert von 5,2 bei FG = 6 eindeutig im 1 ‰-Niveau signifikant, während die Differenz zwischen 2,8 und 4,7 dB bei einem t Wert von 2,05, FG = 6 sicher bedeutsam, aber statistisch als nicht mehr signifikant einzuschätzen ist Die Ausflußöffnung des Perfusionsmittels wurde in 3–4 mm Entfernung vom runden Fenster in der Scala vestibuli angelegt und beeinflußte, da sie stets einen wesentlich kleineren Durchmesser als 0,5 mm hatte, die Höhe der RMP nicht

Der gemessene Abfall der Potentiale kann weder durch Verletzung der Innenohrstrukturen noch durch einen elektrischen Nebenschluß verursacht werden, wie die vorher beschriebenen Versuchsergebnisse zeigten

Daß die Senkung des hydrostatischen Drucks nach Anbohren der Kochlea von normal 3,5 mmHg (Martinez, 1969) auf Null im Perilymphraum keinen Einfluß auf die Höhe der RMP hat, bewiesen unsere Druckversuche

Wir nehmen deshalb an, daß ein Bohrloch in

einer der Scalen, in unserem Falle in der Scala tympani, in seiner Wirkung auf die Hydro mechanik der Schnecke zu einem mehr oder weniger starken akustischen Nebenschluß (abhängig von Lochdurchmesser und Frequenz) in der jeweiligen Scala fuhrt Der Zusammen hang zwischen Bohrlochposition und Auswir kung auf den Frequenzgang des RMP stellt dabei ein sehr komplexes Problem dar, das einigermaßen zuverlässig nur mit Hilfe eines Kochlea Modells (z B Oetinger & Hauser, 1962) und Integration der „gestörten" Basilarmembran-Auslenkung analog Wagner et al (im Druck) durchschaut werden kann Lediglich der Einfluß einer Bohrung in nächster Umgebung des runden Fensters kann unmittelbar abgeschätzt werden Dort hat eine Bohrung dann keine Minderung des RMP zur Folge, wenn ihr Durchmesser ein bestimmtes Maß unterschreitet, weil dann die Bohrloch-Impedanz gegenüber der des runden Fensters vernachlassigt werden kann Dies wird durch Abb 1 bestätigt

Die Ergebnisse von Jung (1974), der bei Druckwerten oberhalb 10 cm Wassersäule einen deutlichen, oberhalb von 25–30 cm Wassersäule einen eindrucksvollen Potentialabfall fand, konnten wir nicht bestätigen

Butler & Honrubia (1963) hatten bereits festgestellt, daß eine Druckerhohung auf 10 cm Wassersäule in der Scala tympani von keiner nennenswerten MP-Änderung begleitet ist Bis *zu Werten von +30 cm bzw −30 cm Wasser*säule konnten wir, von einigen Ausnahmen abgesehen, die Höhe der RMP konstant halten Erst dann kam es am ovalen Fenster (Ringband) oder am runden Fenster gehauft zu Rupturen, die die RMP absinken ließen Wir betrachten diese Schäden als Folgen der Praparation am Ringband und des Anlegens der Elektrode am Rand des runden Fensters mit moglicher nachfolgender Schädigung weiterer Innenohrstrukturen Der nach Moscovitch et al (1973) relativ weite Aquaeductus cochleae (Durchmesser 185–200 μm) und die dauernd vorhandene Druckdifferenz von 1 mmHg zwischen Liquorraum (4,5 mmHg) und Perilymphraum (3,5 mmHg Martinez, 1969) zwingen bei Beachtung der Ergebnisse von Arnold & v Ilberg (1972) zu der Annahme eines dauernden Liquorstroms aus dem Aquaeductus cochleae uber das runde Fenster in den subepithelialen Raum der Mittelohrschleimhaut Wie diese Verhältnisse durch die Druckerhohung bei der Perfusion verändert werden, sollten Untersuchungen mit durch Patentblau gefarbtem Perfusionsmittel zweigen Bis zu einem Perfusionsdruck von etwa 0,5 bis 1 cm unter 5,6 cm Wassersäule wurde Perilymphe bzw Liquor in die Kapillare gedruckt Der Perilymphdruck lag dann uber dem des Perfusionssystems (Abb 2*a*)

Bei +5,6 cm Wassersäule begann die angefärbte Flussigkeit in die Kochlea und den Liquorraum einzufließen (Abb 2*c*) Nach den Druckwerten von Martinez (1969) und den Ergebnissen von Arnold & v Ilberg müßte man annehmen, daß bei einem Perfusionsdruck zwischen +4,7 und +6,1 cm Wassersäule die einströmende Flüssigkeit über das subepitheliale Gewebe des runden Fensters wieder abfließt und erst bei Überschreiten des letzten Wertes auch in den Liquorraum gelangt Mit unserer relativ trägen und nicht sehr genauen Methodik konnten wir dies bestatigen, da zwischen +4 6 bzw +5,1 und +5,6 cm Wassersäule eine Phase relativer Ruhe bzw sehr langsamer Einflußbewegung erreicht wurde (Siehe Abb 2*b*) Das spricht fur die Richtigkeit des oben beschriebenen Mechanismus, wie auch der angegebenen *Druckwerte Den von Weille et al* (1958) gemessenen Perilymphdruck von 24 mmHg können wir nicht bestätigen

Fur die Perfusion ergeben sich aus diesen Ergebnissen folgende Konsequenzen

1 Bei einem Perfusionsdruck unter +5,6 cm Wassersäule wird das Perfusionsmittel, mit abnehmendem Druck vermehrt, durch ein fließenden Liquor verdünnt, wobei ein maximaler Zuflußwert von 0,5 bis 1 μl/min gemessen wurde
2 Man muß berucksichtigen, daß mit steigen dem Druck eine zunehmende Menge des Perfusionsmittels in das subepitheliale Gewebe des Mittelohrs abfließt
3 Beim Überschreiten des Drucks von +5,6

cm Wassersäule fließt zunehmend Perfusionsmittel in den Liquorraum

Die Variation der Perfusionsgeschwindigkeit zwischen 1 und 50 μl/min hatte nach unseren Untersuchungen keinen Einfluß auf die Funktion des Cortischen Organs, wenn nicht durch Erhöhung des Drucks Innenohrstrukturen geschädigt wurden

SUMMARY

The decrease in microphonic potentials detected from the round window (RMP) is negligible only if the bores are located very close to the round window, whereas it will increase with increasing distance from it. Only at pressures of more than ±30 cm water column, in rare cases even below that, will a sharp decrease in the RMP occur, possibly because of a lesion of either the oval or the round window membrane At a perfusion pressure above +5 6 cm water column, the perfusion liquid will enter the cerebrospinal fluid space At a lower pressure the perfusion liquid is diluted by cerebrospinal fluid via the cochlear aqueduct The perfusion speed of 1 to 50 μl/min has no effect on the function of the organ of Corti

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Für die Perfusion ergeben sich aus diesen Ergebnissen folgende Konsequenzen

1 Bei einem Perfusionsdruck unter +5,6 cm Wassersäule wird das Perfusionsmittel, mit abnehmendem Druck vermehrt, durch ein fließenden Liquor verdünnt, wobei ein maximaler Zuflußwert von 0,5 bis 1 μl/min gemessen wurde
2 Man muß berücksichtigen, daß mit steigendem Druck eine zunehmende Menge des Perfusionsmittels in das subepitheliale Gewebe des Mittelohrs abfließt
3 Beim Überschreiten des Drucks von +5 6

cm Wassersaule fließt zunehmend Perfusionsmittel in den Liquorraum

Die Variation der Perfusionsgeschwindigkeit zwischen 1 und 50 μl/min hatte nach unseren Untersuchungen keinen Einfluß auf die Funktion des Cortischen Organs, wenn nicht durch Erhohung des Drucks Innenohrstrukturen geschadigt wurden

SUMMARY

The decrease in microphonic potentials detected from the round window (RMP) is negligible only if the bores are located very close to the round window whereas it will increase with increasing distance from it Only at pressures of more than ±30 cm water column, in rare cases even below that will a sharp decrease in the RMP occur, possibly because of a lesion of either the oval or the round window membrane At a perfusion pressure above +5 6 cm water column the perfusion liquid will enter the cerebrospinal fluid space At a lower pressure the perfusion liquid is diluted by cerebrospinal fluid via the cochlear aqueduct The perfusion speed of 1 to 50 μl/min has no effect on the function of the organ of Corti

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is due to a sensory loss (Clemis et al, 1967, Davies, 1968) rather than eighth nerve entrapment as postulated in an early report (Nager, 1919)

The mechanisms of the sensorineural loss may include vascular shunts and degenerative changes Ruedi (1968) has shown evidence that vascular shunts can develop between the normal inner ear circulation and the pathological circulation in the Pagetic bone replacing the otic capsule and surrounding the eighth nerve at the internal auditory meatus These shunts could produce stasis and anoxia in the stria vascularis with consequent dysfunction of the spiral organ However, no vascular shunts were seen by Kornfield (1967), Davies (1968), or Lindsay & Lehman (1969) in their temporal bone studies Kornfield (1967) examined seven temporal bones affected by Paget's disease and noted that replacement of the endosteal layer of the otic capsule with Pagetic bone produced degenerative changes in the stria vascularis Degeneration of the spiral organ has not been a prominent feature in the temporal bones studied

Vertigo has not been a prominent symptom in clinical reports and caloric testing has shown a low incidence of vestibular dysfunction (Davies 1968, Clemis et al, 1967)

Treatment of Paget s disease with synthetic human calcitonin is associated with clinical, biochemical, histological and radiological improvement (Woodhouse et al, 1971, Greenberg et al, 1974)

The purpose of the present study was to investigate the type of hearing loss that occurs in patients with Paget s disease of bone, and to determine the effect of short term calcitonin treatment on hearing loss considered to be due to this disease

PATIENTS AND METHODS

From a group of 38 patients with painful Paget s disease accepted for treatment with synthetic human calcitonin at Hammersmith Hospital, 8 patients complained of hearing impairment Two proved to have chronic suppurative otitis media and one had essentially normal hearing and these 3 patients were not investigated further

The remaining 5 form the group studied in the present series There were 3 males and 2 females and their ages ranged from 50 to 64 years One patient (L M) had had a stapedectomy on her left ear a year previously and one (J A) had a history of acoustic trauma The remaining 3 patients had normal drums with no history of drug exposure, head injury, or other causes of deafness All five had been deaf for longer than a year, one patient (N R) having been deaf for 20 years Vertigo had also been noted by 2 patients (N R, J A) and tinnitus by another (L E) The diagnosis of active Paget's disease of the skeleton was established by the typical radiographic appearances and by the findings of markedly increased serum alkaline phosphatase concentrations (range 105 to 363 KAU), and urinary total hydroxyproline output (range 151 to 1 245 mg/day)

The 5 patients were investigated, before commencing calcitonin treatment, by pure tone audiometry, Carhart Tone Decay Test, Bekesy audiometry, by acoustic impedance, using the Madsen Z070 Electro-acoustic impedance bridge, and by radiological assessment of the temporal bone by tomography using the Massiot Polytome All 5 patients were then followed by pure tone audiometry Three (L E, N R and J A) were treated with synthetic human calcitonin, 0 5 mg twice daily, by intramuscular injection for 18 to 22 months One patient (L M) was given 0 5 mg weekly for 12 months and then 1 mg daily for 8 months One (L B) remained untreated during this study

RESULTS

Pre-treatment Findings

(a) Pure tone audiometry (Table I)

The data in Table I show that there was sensorineural loss in 4 patients (L B, L M, N R and L E) in that the bone conduction thresholds exceeded the published norms of Hinchcliffe (1959) The sensorineural loss was most

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OTOLOGICAL STUDIES IN PATIENTS WITH DEAFNESS DUE TO PAGET'S DISEASE BEFORE AND AFTER TREATMENT WITH SYNTHETIC HUMAN CALCITONIN

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Abstract Audiometric and radiological assessments of 5 patients with deafness due to Paget's disease of the skull are presented It was found that all patients had a mixed deafness The conductive element was demonstrated in all ears by both negative Rinne tests and by pure tone audiometry A variety of radiological changes was found in the middle ear in all cases, and all ears had abnormal tympanometry Sensorineural loss was shown to be limited to end organ disease Four patients were then treated for 18 to 22 months with synthetic human calcitonin with an improvement in those biochemical parameters which reflect skeletal disease No improvement in the hearing occurred

Although the association between Paget's disease of the skull bones and deafness has been recognised since the original description of the disorder by Paget (1877), the exact pathogenesis of the hearing loss is obscure The deafness has usually been reported as mixed, but in some patients apparently pure conductive or sensorineural loss may occur (Mayer, 1913, Nager, 1919, Jenkins, 1928, Brunner, 1931, Lindsay & Perlman, 1936, Clemis et al, 1967 Davies, 1968)

In Paget's disease, lamellar bone is replaced by highly irregular calcified and woven bone, and these changes have also been reported in affected temporal bones Earlier studies, based on 42 temporal bones (Mayer, 1917, Jenkins, 1933, Anson & Wilson, 1937, Tamari, 1942, Waltner, 1965, Kornfield, 1967, Ruedi, 1968, Davies, 1968, Lindsay & Lehman, 1969), have shown that the process seems to begin at the apex of the petrous pyramid and then extend laterally, to eventually involve the entire otic capsule The capsule is relatively resistant, histological studies indicate that, when involved, changes initially occur in the periosteal layer and then pass inwards to involve the endosteal layer

The conductive loss that occurs in Paget's disease has usually been found to affect the lower frequencies and has been related to the following changes in the middle ear

1 Fixation of the stapedial footplate This was only found in three of the above 42 temporal bone studies, where it was due to bony involvement of the footplate in two and to thickening of the annular ligament in one

2 *Enlargement of the ossicles* (Davies, 1968)

3 Bony spurs in the attic impinging on the ossicles (Davies, 1968)

In some patients with deafness, however, none of these middle ear abnormalities are present and the poor results of surgical treatment by stapedectomy, which attempts to correct one of these factors (*Waltner, 1965, Davies, 1968*) suggests that other mechanisms may also be relevant

In contrast to the conductive loss, the sensorineural loss is usually most pronounced in the higher frequencies (Mayer, 1913, Nager, 1919, Jenkins, 1928, Brunner, 1931, Lindsay & Perlman, 1936, Clemis et al, 1967, Davies, 1968) Testing of eighth nerve function suggests that it

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is due to a sensory loss (Clemis et al, 1967, Davies, 1968) rather than eighth nerve entrapment as postulated in an early report (Nager, 1919)

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Table I *Pure tone audiograms before treatment, hearing threshold levels in decibels (1964 I S O values)*

Patient			250	500	1 000	2 000	4 000	6 000	8 000
			Frequency, Hz						
L B	R	bc	5	10	20	35	40		
		ac	30	30	45	45	50	55	55
	L	bc	0	5	5	15	30		
		ac	10	10	5	15	40	40	50
L M	R	bc	5	25	40	55	5		
		ac	65	65	65	65	30	15	50
	L*	bc	10	5	25	40	25		
		ac	40	25	35	40	35	50	60
N R	R	bc	25	20	40	50	55		
		ac	65	75	85	105	100	95	95
	L	bc	20	25	30	55	45		
		ac	70	85	90	100	80	90	90
L E	R	bc	10	−5	5	10	30		
		ac	30	30	30	20	30	50	60
	L	bc	−10	10	10	25	60		
		ac	40	45	50	45	80	75	70
J A	R	bc	−5	−5	5	5	30	20	30
		ac	20	10	5	5	30	20	30
	L	bc	−5	−5	−5	−5	40	10	0
		ac	40	40	−5	−5	40	10	0

R = right, L = left ac = air conduction bc = bone conduction, * = stapedectomy previously

marked in the higher frequencies in six of the ten ears tested

All ears showed an air–bone gap, and in of the ten ears tested, the air–bone gap maximal in the lower frequencies The air bone levels were separated by an average of 33 dB at 250 Hz, 23 dB at 1 000 Hz and 15 dB at 2 000 Hz L M, who had previously had a stapedectomy in the left ear, showed a smaller air–bone gap in this ear than in the right ear Preoperative audiometry is not available but the operative note states that the middle ear changes were morphologically similar to those of otosclerosis and that the footplate was fixed Histological examination of the stapes did not show either otosclerosis or Paget's disease The patient reported some improvement in hearing in this ear following stapedectomy J A showed a high tone notch maximal at 4 000 Hz in both ears which was compatible with the history of acoustic trauma

(*b*) *Carhart tone decay test* (Table II)

The results of this test were compatible with the sensorineural loss, being limited to the cochlea in all patients apart from L M who had marked tone decay in both ears, indicating a neuronal element in her deafness

(*c*) *Bekesy audiometry*

Two of the patients tested (J A and L E) showed Jerger (1960) type I graphs in both ears and the remaining 3 showed type II graphs in both ears

(*d*) *Acoustic impedance studies* (Table III)

In five ears, the compliance was below the lower limit of normal (Brooks, 1969), and in

Table II *Carhart Tone Decay test before treatment*

Patient	Right ear	Left ear
L B	20 dB (1 000 Hz)	5 dB (1 000 Hz)
L M	110 dB (4 000 Hz)	65 dB (2 000 Hz)
N R	Not recordable	30 dB (4 000 Hz)
L E	10 dB (4 000 Hz)	10 dB (4 000 Hz)
J A	20 dB (4 000 Hz)	10 dB (4 000 Hz)

Table III *Acoustic impedance studies before treatment*

Patient	(cc)	Compliance (cc)	Middle ear pressure (mm H_2O)	Stapedius reflex
L. B	R	0 37	−85	−
	L	0 38	0	−
L. M	R	0 37	−85	−
	L	0 60	−80	−
N R.	R	0 20	−70	−
	L	0 47	−20	−
L. E.	R	0 30	−40	−
	L	0 09	0	−
J A	R	0 13	50	+
	L	0 25	−120	+

every ear the compliance fell below the median value of 0 7 cc for normal ears The middle ear pressure of eight ears was negative, while in five ears it fell below the value of −50 mm H_2O, the lower limit for normal ears (Alberti & Kristersen, 1970) Eight ears had a negative stapedius reflex at all frequencies tested A reflex could not be elicited from the left stapedius muscle in L M, this is compatible with a previous stapedectomy on this side J A had positive stapedius reflexes in both ears

(*e*) *Radiological assessment* (Table IV)

All patients had gross disease of both temporal bones

Findings Following Treatment

The 3 patients treated initially with 1 0 mg calcitonin daily showed evidence of biochemical response, in L E, normal serum alkaline phosphatase concentrations and urinary hydro response in L E, normal serum alkaline phosphatase concentrations and urinary hydroxyproline excretion rates were achieved within 10 months and in the other 2, these indices fell to less than 75% (N R) and less than 47% (J A) of the pre treatment values The fourth patient (L M) showed a reduction of serum alkaline phosphatase concentrations to 71% of control levels when being treated with 0 5 mg weekly and a further biochemical response when the calcitonin dose was increased Pure tone audiometry was carried out at intervals in the 4 treated patients but no change occurred in the hearing of any, the duration of the follow-up was 18 to 22 months The untreated patient (L B) also showed no change in his hearing during 14 months of follow-up

DISCUSSION

Structural abnormalities of Paget's disease were apparent from the X-rays of the middle ears of all patients, and the Rinne test and pure tone audiometry showed that all had a conductive deafness The precise pathogenesis of the conductive deafness in these cases is unknown, but it is of interest that in 2 of the patients with unilateral ossicular enlargement (L B and J A), in whom there was no platybasia or gross distortion of the auditory meati, the extent of the air–bone gap was greater on the side of ossicular enlargement Furthermore, the finding of abnormal negative middle ear pressures in five of the ten ears, suggests that there may be auditory tube obstruction, which could be due to occlusion of the lumen by the abnormal bone, or even by dilated blood vessels, thus the effect of placing ventilation tubes in the tympanic membrane could be tried in an attempt to correct this possible cause of conductive deafness The finding of reduced compliances and negative stapedius reflexes, reflect stiffness in the ossicular mechanism, radiological evidence of encroachment on the oval window was found in only 2 patients, however, and alternative mechanism causing stiffness are not excluded by this study

In addition to conductive defects, 4 patients had an associated sensorineural deficit, only one of whom (L M) had any evidence of neural damage on the Carhart Tone Decay Test, however, in view of her showing a type II Bekesy audiogram, the mechanism in her case remains obscure

Comparison of the audiometric findings in each of our 4 patients, before and followin

Table IV. *Radiological changes*

Patient		Platybasia and IAM/EAM distortion	Ossicular enlargement	Sclerosis of otic capsule	Reduction in volume of middle ear	Oval window encroachment
L. B	R		+	+		
	L			+		
L. M	R	+				+
	L	+				+
N R	R	+	+			
	L	+			+	
L E	R	+				
	L	+				
J A	R		+			+
	L					+

calcitonin administration, suggested that no changes had occurred, either in the magnitude or the type of hearing loss, despite the improvement in biochemical parameters The only comparable study we are aware of involved treatment with porcine calcitonin, for periods up to 17 weeks (Shai et al , 1971), improvement in the air conduction component was reported in 3 of 6 patients with hearing loss due to Paget's disease, although the magnitude of the changes was minimal

If the temporal bones are as responsive to synthetic human calcitonin as are other bones, treatment may well be expected to retard the progression and perhaps to improve the conductive hearing loss Our results do not exclude this possibility and further studies to investigate the natural history of the hearing loss, and the effect of more prolonged calcitonin treatment, from an early stage in the disease, are indicated

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We are also indebted to CIBA GEIGY Ltd for support and supplies of synthetic human calcitonin

ZUSAMMENFASSUNG

Audiometrische und radiologische Messungen wurden an fünf Patienten mit Taubheit durch die Pagetsche Krankheit vorgenommen

Es zeigte sich, dass alle Patienten eine gemischte Taubheit aufwiesen Sowohl durch den negativen Rinne-Test als auch durch reine Tonaudiometrie wurde in samtlichen Ohren das leitende Element festgestellt Eine Vielfalt von radiologischen Veränderungen im Mittelohr wurde bei samtlichen Fallen gefunden, und bei samtlichen Ohren zeigte sich eine abnorme Tympanometrie Sensorielle Verluste wurden zur beschränkten Beendigung der Krankheit des Organs erklärt Vier Patienten wurden dann wahrend 18 bis 22 Monaten mit synthetischem Calcitonin behandelt Es zeigte sich eine Verbesserung der biochemischen Parameter, die Knochenkrankheiten angeben, aber keine Verbesserung der Gehorfunktionen

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RECRUITMENT IN CONDUCTION DEAFNESS

Observations Using a Midline Loudness Balance Test

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From the Department of Otolaryngology, University of Oulu, Oulu, Finland

(Received June 11, 1974)

Abstract A midline loudness balance test was made in 10 normal ears with blocked ear canals and in 104 conductively deaf ears with loss not exceeding 45 dB The test was made by giving the tones simultaneously in the ears at 10, 60 and 80 dB sensation levels for 500 and 2 000 Hz In the normal group the midline balance method indicated no reduction from the threshold differences at any of the three levels whereas significant reductions occurred in all groups of conduction deafness The occurrence of true partial recruitment in conduction deafness demonstrated by ABLB (Fowler s) test was attributed to altered middle ear mechanics loud tones stimulating the inner ear relatively more effectively than tones near the threshold level The midline balance method is more closely dependent upon bone conducted unds and on the altered relative movements of the yrinthine windows

e recruitment phenomenon, i e abnormally rapid growth of loudness in perceptively deafened ears, is characteristic of inner ear lesions and absent in most cases of nerve fibre retrocochlear lesions This division is so commonly accepted that the loudness level growth in conduction impairment has been very little studied and the group as a whole is regarded as exemplifying those ears in which no loudness recruitment occurs

Single observations of recruitment in conduction deafness were recorded earlier by Palva & Ojala (1955), using Fowler's test, in 4 ears of 42 patients suffering from catarrhal or purulent otitis media Recruitment was complete in three ears and incomplete in one This recruitment of loudness was apparently not due to inner ear lesions since bone conduction thresholds were either normal or returned to normal after aspiration of fluid

The series of Anderson & Barr (1966) consisted of 30 ears, of which 24 were conductively deaf due to ossicle fixation (18 otosclerotic ears) In the remaining six ears, the loss was due to ossicular interruption In addition to Fowler's test, the stapedius reflex test was recorded from the contralateral normal ear the stimulus being given to the impaired ear In Fowler's test, the fixation group showed partial recruitment and the interruption group practically no recruitment The stapedius reflex test elicited the response in the fixation group from the contralateral healthy ear at 17 to 30 dB lower levels than could be expected from the threshold curves In the interruption group, no such lowering of stapedius reflex level was found

In the series of Rahko from our laboratory, 63 patients with unilateral conduction deafness were examined with the Fowler's alternate loudness balance (ABLB) test Fortythree of them had chronic ear disease and 20 had otosclerosis Hearing loss in the impaired ear did not exceed 50 dB, and the testing was done at 500 and 2 000 Hz None of the patients showed complete recruitment Partial recruitment (final difference at 100 dB SL 10–25 dB) appeared in 23 % of the cases at 500 Hz and in 33 % at 2 000 Hz the majority showed no recruitment In otosclerosis the figures for partial recruitment were 40 and 25 %, respectively

This work was aided by a grant from the Finnish State Medical Research Council

Anderson & Barr (1966) noted that if the fixation group was divided into two groups according to degree of impairment more than or less than 50 dB the former showed greater recruitment Even if the effect of cross hearing was discussed it may have contributed to this difference When average size earphones are used, interaural attenuation does not exceed 50 dB and may in some ears be as low as 40 dB (Palva & Palva, 1962) Evidently, then, one can expect considerable cross hearing in those cases in which the threshold loss measured with appropriate masking is around 60–70 dB In Fowler s balance test this may result in a decrease of some 15–25 dB from the initial thresh old difference

Similarly, the threshold for the stapedial re flex in ears with a greater loss might be due to combined sound effect from both ears, or even from the ear being recorded For instance an ear with a 70 dB threshold loss might give a stapedius muscle contraction from the opposite ear when stimulated with 120 dB intensity A minimum of 40 dB loss across the head would imply that the healthy ear under recording was stimulated by an 80 dB tone The stimulus and recording might then represent this same ear, or a combined hearing effect from both ears Of course this line of reasoning leads to the question whether all these greater losses are in effect cases of mixed deafness, with some 10 to 30 dB inner ear or nerve involvement

Anderson & Barr in their series found partial recruitment more often compared with the data from this laboratory We have therefore dis cussed at some length the data obtained by them to illustrate the complex nature of con ductive recruitment The only way to avoid the cross over effects is to apply stringent criteria for case selection, accepting only those in which the threshold level loss is less than 50 dB and bone conduction within normal range

In this study we have wanted to approach this problem from another angle In everyday hearing, both ears are used simultaneously and our patients have often spontaneously stated that their hearing improvement postoperatively was greater than could be expected from the threshold curves We have, therefore, tested a series of conductively impaired ears with the simultaneous bilateral midline balance test to imitate the conditions prevailing for instance when listening to speech or stronger sounds with both ears

MATERIAL AND METHODS

The series consists of 10 persons with normally hearing ears and 104 patients belonging to vari ous groups of conductive deafness, all of whom had normal hearing in the contralateral ear It is classified into 6 groups, as follows

Group I	Normal ears	10
Group II	Ossicular defect	9
Group III	Drum defect	23
Group IV	Otosclerosis	20
Group V	Adhesive otitis	20
Group VI	Chronic otitis	32

All patients with an ossicular defect had a normal or healed drum membrane and a surgically verified bone defect of the long process of incus or of the stapes superstructure How ever, among the cases with partial incus loss, there were fibrous soft tissue strands from the remaining part of incus to the stapes head The patients with drum defects had perforations of varying size, but at surgery the ossicular chain was found to be intact All cases of otosclerosis had a rigid footplate and were verified surgically In the group of adhesive otitis the drum was adherent to the promontory and there was no middle ear air space The group of chronic ears included cases discharging continuously or intermittently and all had marked middle ear changes with a drum defect and often ossicular loss Bone conduction values were normal in all cases

One ear of the normally hearing persons was filled tightly with wax prior to testing Pure tone testing was made with a Madsen 70 audiometer using the manual method of limits with the descending ascending technique Masking was employed in bone as well as air conduction

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Table II *Frequency of complete recruitment using midline balance in various test groups*

	Total cases	Complete recruitment	Per cent
Group I	10	—	0
Group II	9	2	22
Group III	23	4	17
Group IV	20	7	35
Group V	20	5	25
Group VI	32	14	43

other hand the averages obtained at 60 and 80 dB SL did not differ significantly from one another

In all other groups listed in Table I, the initial threshold difference became still smaller varying from −14 to −23 dB at 500 and 2000 Hz All these reductions in loudness level were statistically highly significant ($p<0\ 005$) In none of the groups at either frequency was there any statistically significant change for the midline balance readings at 60 to 80 dB SL ($p>0\ 05$)

The frequency of ears with complete recruitment in the above test groups is shown in Table II The greatest incidence, 43 %, was found in the group of chronic otitis

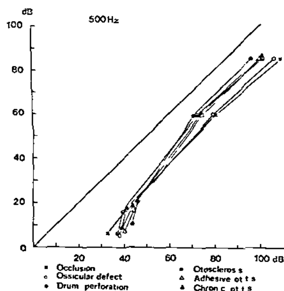


Fig 1 Average results of loudness level comparison using midline balance test at 500 Hz. The occlusion group shows no recruitment

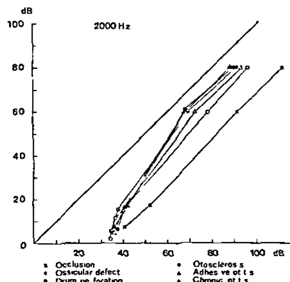


Fig 2 Results of midline balance test at 2 000 Hz. The occlusion group is more clearly separated from the others, compared to the results at 500 Hz.

Figs 1 and 2 show graphically the results in the standard form used for loudness level comparisons Studied by midline balance, there appears a clear tendency for the average values to show partial recruitment in all the clinical groups of conduction deafness studied the values being slightly larger for 2 000 Hz than for 500 Hz However, statistically there was no difference between these two frequencies in any of the numerous comparisons made

Fig 3 shows the typical non recruiting type of result obtained in one normal person with an occluded ear canal Fig 4 illustrates a case of otosclerosis showing complete midline loud-

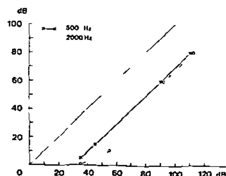


Fig 3 Results in a normal hearing subject with one ear canal artificially occluded. There is no recruitment.

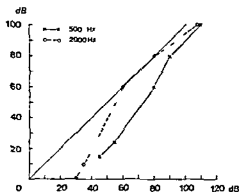


Fig 4 Results in individual case of unilateral otosclerosis At 2 000 Hz a full equality is obtained between 60 and 80 dB SL, the curves parting again at 100 dB SL. The curve for 500 Hz is of a straight line type

ness balance at 60 dB SL for 2 000 Hz, and a different slower type of slope at 500 Hz Fig 5, representing a case with chronic ear disease, shows a similar response but the frequency dependence is reversed

DISCUSSION

The method of midline localization is often considered to be less exact than the ABLB ethod for making loudness comparisons In s series the midline balance was easily obined within the limits of ±5 dB and this was similar to the result we had earlier recorded with the same type of test (Palva, 1955) Nevertheless, the midline simultaneous balance is different from ABLB test since a judgment of

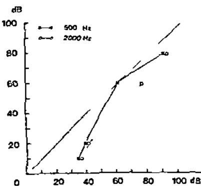


Fig 5 Results in an individual case of unilateral chronic ear disease At 500 Hz a full equality is reached at 60 dB SL, *but the curves separate* again at 80 dB SL. The slope of the curve at 2 000 Hz is more gradual

central localization is here substituted for loudness balance judgment Using tones exceeding 50 dB, inertia and compression bone conduction mechanisms begin to play an important part in the subjective sensation of localization, directly comparable to Weber's test

The midline localization test showed in all groups with true conduction deafness, located in the structures between the drum and stapes footplate, a distinct reduction of the initial threshold differences On the other hand, if the ear canal was artificially blocked with wax, causing an average conduction deafness of 26 dB at 500 Hz and 33 5 dB at 2 000 Hz, the *midline balance tests showed insignificant differ*ences compared with the threshold levels at 10, 60 and 80 SLs in all tests The tests on patients with true conduction deafness revealed another interesting phenomenon A growth of loudness more rapid than normal, was observed in the diseased ear at 10 and 60 dB SL often with an asymptotic type of slope, the average loudness level growth then levelled off and in no group was there a significant difference between the average test results at 60 dB and 80 dB SL In other words, the midline balance showed ' loudness recruitment" during the first 60 dB SLs above normal threshold, amounting on an average to some 10–20 dB Individually, however, the differences could be marked, in each group there were some cases with complete recruitment and some with none

This finding agrees in the main with our own earlier observations (Palva & Ojala, 1955) and with those discussed in greater detail by Anderson & Barr (1966) A major difference as compared with the data of Anderson and Barr is that their group of 6 cases with interruption of the ossicular chain behaved in the same way as our blocked canal group, whereas our corresponding group showed significant reduction in SLs of the poor ear in the midline balance test The differences in testing method, however, may account to this difference

The present data showed considerably more "recruitment" than reported in similar patient material by Anderson & Barr (1966) and by

Rahko (1974) It seems clear that the midline balance method is responsible for this difference Indeed, we made a comparative study of 10 further patients using both ABLB test and mid line balance, the former method consistently showed much less recruitment than the latter

The results obtained by the ABLB test (Rahko, 1974) indicated that partial loudness recruit ment occurs in a significant number of patients 20 to 40 %, depending upon the cause Hearing loss in all these patients was less than 50 dB, safeguarding against cross hearing effects It seems to us that this loudness increase is due to the response of the altered drum–ossicle com plex to stimuli of varying intensity Once the delicate balance of an intact conductive mech anism has been altered then the suprathreshold tones may overcome either the stiffness of the mass of the ossicles more effectively than our logarithmic scale presupposes for normal loud ness level increases

The midline balance, on the other hand is not in fact a measure of loudness recruitment in the proper sense of the word In addition to the additive effect of loudness level in general when both ears are stimulated simultaneously, it obviously is largely dependent upon the inertia and compression bone conduction, and thus of altered relative movements of the laby rinthine windows If the oval window is relative ly fixed and the round window free, a tone sufficiently strong to be also conducted by bone causes an apparent subjective loudness recruit ment in the poor ear The midline balance may then be obtained even at the same SLs in both ears This finding explains the subjective sensa tions of improved hearing and localization of those conductively deaf persons who may have considerable threshold losses

ZUSAMMENFASSUNG

An 10 normalhorenden Ohren mit blockiertem Ohren kanal und 104 Ohren mit Leitungsschwerhorigkeit deren Horverlust nicht 45 dB uberstieg, wurde ein Mitten lokalisations Lautheitsvergleichstest durchgefuhrt Bei der Untersuchung wurde bei den Testfrequenzen 500 Hz und 2 000 Hz der Ton jeweils gleichzeitig in beiden Oh ren bei Horpegeln von 10 60 und 80 dB SL gegeben In der Gruppe der Normalhörenden zeigte die Mitten lokalisationsmethode bei keinem der drei Horpegel eine Minderung in der Pegeldifferenz im Vergleich zu der durch die Blockierung bewirkten Horschwellendifferenz bei allen Gruppen mit Leitungsschwerhorigkeit dagegen wurden signifikante Verminderungen in den Pegeldiffe renzen gefunden Das Vorliegen von partiellen Recruit ment bei Leitungsschwerhorigkeit das durch Fowlers Lautheitsvergleichstest (wechselseitigen Lautheitsab gleich) nachgewiesen wurde wurde einer veranderten Mechanik des Mittelohrs zugeschrieben derzufolge laute Tone das Innenohr relativ effektiver als Tone in Schwel lenpegelnahe stimulieren Bei der Mittenlokalisations methode wirkt in starkeren Grade der durch Knochen le tung uberfuhrte Schall und die veranderte relative Bewegl chkeit der Fenster des Innenohrs ein

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EXPERIMENTALLY INDUCED FACIAL NERVE COMPRESSION IN CATS

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Abstract An experimentally controlled compression of the facial nerve was induced in 32 cats by applying polyethylene tubes of different diameter on its main trunk The effect of decompression (removal of the compressing [illegible] gation of the nerve specimens The results obtained [illegible] jury The release of circumferential pressure over the degenerated facial nerve within the above mentioned delay is thus beneficial for optimal regeneration The possible implications of these findings for the treatment of Bell s palsy are discussed

In spite of the refinements of electrodiagnosis of facial nerve injuries (May et al , 1972, Esslen, 1973), there is no method to determine by electrical stimulation whether endoneural tubes of degenerated nerve fibers are still intact or not This is an unfortunate situation since the end result of regeneration depends entirely upon the condition of these latter structures Intact endoneural tubes ensure a good recovery of function (*a*) by guiding the regenerating axons into their original path and thus avoiding haphazard intermingling of axons with resulting mass innervation, and (*b*) by avoiding the loss of regenerating nerve fibers at the site of injury

This study was undertaken in order to investigate whether preservation of endoneural tubes can be obtained by releasing circumferential compression acting upon degenerated facial nerve fibers

MATERIAL AND METHODS

A total of 32 cats (weighing 3–3 5 kg) were used in this study Preliminary experiments on 14 animals were necessary to ascertain the best method for circumferential compression of the facial nerve The chosen method consisted of the application of polyethylene tubes (Ulrich Co , St Gallen) around the main trunk of the facial nerve The nerve trunk was exposed in the anesthetized animal (Nembutal, Abbott) and compressed 2 mm proximal to its bifurcation Two tube diameters each measuring 3 mm in length were used One tube had an inside diameter approximately equal to that of the facial nerve and the other an inside diameter approximately 2/3 of that of the nerve These were tightly applied around the exposed nerve trunk by two stainless steel wires measuring 100 μm in diameter (Figs 1, 2)

Decompression of the nerve was carried out by releasing the wires, removing the tube and incising the epineural sheath at varying intervals between 5 to 28 days after surgery The condition of the nerve fibers was assessed at decompression by electrical stimulation In all instances complete degeneration (no eye movements and absence of summating potentials) was found after maximal stimulation (1 0 msec, 80 V) of the nerve 5 mm distal to the compression site

Recovery of facial movement was assessed over a period of 20 weeks (1) by observation

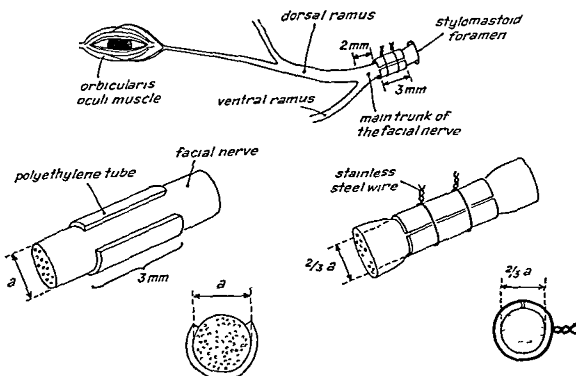


Fig 1 Surgical technique used for nerve compression a = outside diameter of the facial nerve $2/3\,a$ = inside diameter of the polyethylene tube used for compression

of the eye blinking reflex, and (2) by recording the summating potentials of the orbicularis oculi muscle

The following grades of eye blinking reflex were distinguished

grade 0 no movement of eyelids (no recovery)
grade 1 incomplete eyelid-closure (poor recovery)
grade 2 complete but weak eyelid-closure (good recovery)
grade 3 complete eyelid-closure (very good recovery)

The summating potentials of the orbicularis oculi muscle were used as an objective measure of the end result of regeneration Stimulation of the facial nerve directly 5 mm distal to the compression site with square waves of 0 1 msec duration and the prior mentioned intensity was carried out Orbicularis oculi response was measured via monopolar surface electrodes applied to the muscle Similar recordings were made before compression and 20 weeks later when the animal was sacrificed The amplitude of the summating potential obtained after the 20 weeks compression was compared with that measured pre compression The ratio between these two amplitudes in percent was taken as measure for the final amount of recovery (Esslen, 1973, Yamamoto & Fisch, 1974)

Specimens of the nerve were removed at the end of the experiment 5 mm distal to the compression site and fixed with osmic acid for histological evaluation

RESULTS

The effect of the application of polyethylene tubes having an inside diameter equal to the outside diameter of the facial nerve was assessed in 8 cats (Table I) The eye blinking reflex remained normal during the entire observation



Fig 2 Surgical site after compression of the main trunk of the facial nerve Note the stainless steel wires used for fixation of the tube

period of 20 weeks in 4 animals In the remaining 4 cats a temporary paresis of delayed onset occurred 4 to 10 days after surgery and disappeared within one week In all instances normal facial movements (grade 3 eye blinking reflex and summating potentials ranging from 86 to 99% of the initial value) were observed at the end of the experiment

The results obtained in 7 cats by compressing the facial nerve with polyethylene tubes having an inside diameter 2/3 of that of the VII nerve are shown in Table II All animals were noted to have a complete paralysis of the facial nerve, 6 within 24 hours, one (cat 25-73) with a delay of 5 days The paralysis of the face did not recover in 3 cats An incomplete recovery of facial movements (summating potential not exceeding 50% of the initial value) was seen in the other 3 animals Only in one instance (cat 36-73) a very good eyelid-closure (grade 3) and a summating potential measuring 68% of the initial value were reached, after a considerable delay of 96 days

Table III shows the results obtained by releasing the facial nerve from the compression produced by the polyethylene tubes 5 to 28 days after the initial surgery All cats had had a complete facial paralysis within 24 hours after compression Five animals decompressed within 12 days after application of the tubes reached a complete recovery of the eye blinking reflex within 34 days The summating potentials of the

Table I *Function of the orbicularis oculi muscle following application of polyethylene tubes having the same diameter as that of the facial nerve (8 cats)*

	Function of the orbicularis oculi muscle		
		At the end of observation period (20 weeks)	
Cat no	During observation period	Grade of eye blinking	Summating potential (% of initial value)
9-73	Temporary paresis	3	99
57-73	Temporary paresis	3	98
27-73	Normal	3	96
5-73	Normal	3	95
36-73	Temporary paresis	3	94
65-73	Temporary paresis	3	93
7-73	Normal	3	93
59-73	Normal	3	86

Table II *Function of the orbicularis oculi muscle after compression of the facial trunk with polyethylene tubes (7 cats)*

Cat no	Op side	Time interval (days) to		Function of the orb oculi muscle at the end of observation period	
		Initial recovery	Maximal recovery	Grade of eye blinking	Summating potential (%)
36–73	R	87	96	3	68
35–73	R	84	90	1	50
25–73	R	88	95	2	36
26–73	L	83	97	2	37
33–73	R	∞	∞	0	18
30–73	R	∞	∞	0	10
50–73	R	∞	∞	0	0

∞ = no recovery at the end of observation period

orbicularis oculi muscle of these cats ranged from 91 to 93% of their initial values Poorer results were obtained when decompression was performed 14 to 28 days after application of the tubes In fact, 3 cats decompressed after a delay of 14 to 21 days presented with a very good clinical recovery (grade 3 eye blinking reflex) but with summating potentials measuring only 54, 72 and 68% of the respective initial values Two of these animals (cat 26–73, 24–73) also developed synkinesis at the end of the observation period The poorest results were seen in the 2 cats decompressed 28 days after initial surgery In both animals eye movements and summating potentials were not different from those observed in the group of non decompressed animals (Table II) Histologic study of nerve specimens removed 5 mm distal to the compression site confirmed the observed functional results The quality of regeneration decreased after a compression lasting for more than 14 days The normal bimodal dispersion of nerve fibers (Gutman & Saunders, 1943, Bischoff, 1965) was progressively lost after compression lasting 14 days or more (Fig 3)

DISCUSSION

The results obtained with the present study show that decompression does improve the quality of regeneration of facial nerve fibers in the pre sence of complete degeneration However, in order to be effective decompression has to be performed within well defined time limits The

Table III *Function of the orbicularis oculi muscle after decompression of the facial nerve (10 cats)*

Cat no	Op side	Time interval (days) to			Function of the orb oculi muscle at the end of observation period	
		Decom pression	Initial recovery	Maximal recovery	Grade of eye blinking	Summating potential (%)
33 73	L	5	24	34	3	93
36–73	L	6	21	29	3	93
25 73	L	7	19	28	3	93
30–73	L	11	14	32	3	92
35–73	L	12	18	24	3	91
26–73	R	14	22	31	3	54
6–73	R	14	23	35	3	72
24–73	L	21	23	47	3	68
23 73	L	28	31	46	1	46
50–73	L	28	32	46	2	39

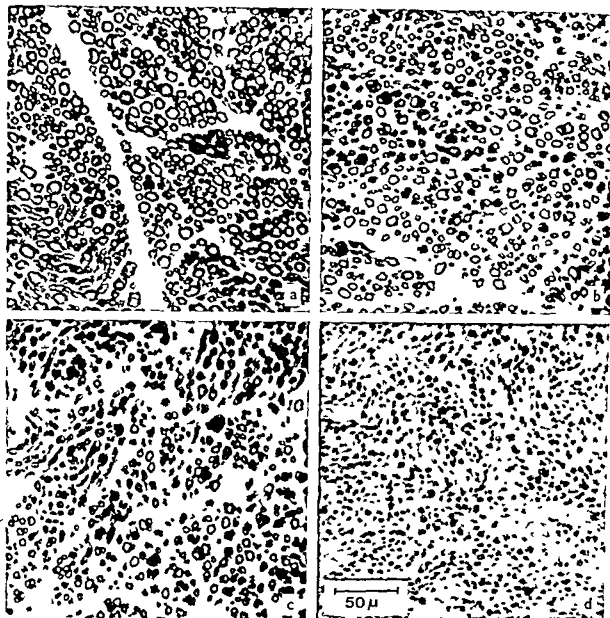


Fig 3 Cross sections of facial nerve specimens removed 5 mm distal to the compression site at the end of the experiment (osmic acid stain) *(a) no compression (normal control)* (*b*) decompression after 5 days (cat 33–73) (*c*) decompression after 14 days (cat 26–73) (*d*) decompression after 28 days (cat 23–73) Note the progressive decrease of the largest myelinated fibers (i e the progressive loss of normal bimodal distribution of facial *nerve fibers) according to the duration of compression* No large fibers are seen when decompression is performed after a delay of 28 days (*d*)

removal of compressing tubes within 12 days of their application leads to a delayed but complete clinical and electrical recovery of the paralysed orbicularis oculi muscle An incomplete recovery as noted in the partial recovery of summating potentials, as well as the appearance of synkinesis, is observed if the release of com*pression is performed 14 to 21 days after the* initial surgery Decompression taking place after a delay of 28 days has no effect on the end results of regeneration

The clinical, electrical and microscopic parameters of good quality regeneration indicate that endoneural tubes are preserved intact if

decompression is performed within 12 days of the compression surgery On the other hand, partial or total disruption of the endoneural tubes are noted to occur when compression lasted for 14 days or more

Rosenberg & Alford (1966) investigated the value of decompression in dogs by releasing a clamp tightened by means of two stainless steel screws over the auriculopalpebral branch of the facial nerve Although the study was mainly concerned with preventing axonal degeneration the results obtained in 12 dogs decompressed when the facial nerve was already degenerated are also given Three of the 4 animals decom pressed 2 weeks after injury had a delayed but complete clinical return of motor function Two of these animals presented with synkinesis The fourth dog had an incomplete return of func tion Eight other animals were decompressed 3 weeks after injury (compression by partial pressure) Although clinical recovery was com plete in all instances the time of recovery was slower in these animals than in those decom pressed earlier Two of these dogs also developed synkinesis These results are in complete agree *ment with ours and confirm that in order to* avoid disruption of the endoneural tubes (syn kinesis) compression of the nerve should not last longer than 14 days

Experimentally induced compression of the facial nerve with polyethylene tubes cannot be directly compared to the clinical situation occur ing in Bell s palsy However, in view of the good quality of regeneration obtained by decom pression of degenerated axons within 12 days the question can be raised whether in Bell s palsy early decompression of degenerated nerve fibers could avoid disruption of endoneural tubes and the subsequent development of dis turbing mass facial movement

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ZUSAMMENFASSUNG

Der Stamm des Nervus facialis wurde bei 32 Katzen mit Polyethylen Rohrchen verschiedenen Durchmessers komprimiert Der Lidschlussreflex sowie die Aufzeich nung des Summationspotentiales des Musculus orbicul aris oculi und die histologische Untersuchung von Ner venanteilen dienten zur Beurte lung des Efffektes der Dekompression (*Entfernung der Kompressionsrohrchen*) Die erhaltenen Resultate zeigen dass die Endoneural rohren der komprimierten Nerven erhalten werden kon nen wenn die Kompression nicht länger als 12 Tage dauert. Die Entfernung des circumferentiellen Druckes uber den degenerierten Facialisnerven innerhalb der 12 Tagesgrenze kann somit gunstig auf die Regeneration der Nervenfasern wirken Die mogliche Bedeutung dieser Feststellung fur die Behandlung der Bell schen Parese wird besprochen

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THE OXYGEN TENSION IN TRANSUDATE OF THE MIDDLE EAR

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Abstract The pO_2 of transudate in the middle ear was measured *in situ* by a small pO_2-electrode introduced through the tympanic membrane Errors involved in some previous methods using aspiration of gas from the middle ear were avoided by the present method since contamination by air from outside of the drum was almost completely eliminated Seventeen patients, of whom the majority had a naso pharyngeal carcinoma or chronic otosalpingitis were investigated The mean pO_2 of the middle ear transudate was 46 mmHg or 6 5 % with a pO_2 range between 10 and 108 mmHg Investigations of the function of the Eustachian tube by the aspiration-deflation method after application of a transmyringeal tube was performed in 10 of the patients, revealing no passage through the Eustachian tube Among these 10 patients only 4 had a passage of air on Politzer, and these had a higher pO_2 in the middle ear transudate than remaining 6 patients in whom Politzer was performed a negative result

Differing opinions about the pathogenesis of middle ear transudate have been published The oldest and perhaps still most widely accepted idea is the theory of *hydrops ex vacuo* based on gas absorption after obstruction of the Eustachian tube

Gas absorption in closed cavities has been studied most thoroughly in subcutaneous gas pockets An absorption of all gases—oxygen, carbon dioxide and nitrogen—occurs in such a closed cavity with soft walls (Rahn & van Liew, 1955) When the cavity has rigid walls a transudation may occur as a result of a decrease in manometric pressure Flisberg et al (1963) showed experimentally that a transudation appeared at a pressure of 20 to 30 mmHg (relative atmospheric pressure) in the cell system of the ear

A few studies of the *gas tension in middle ears during normal conditions* have been published Melville Jones (1961) calculated the contents to be 9 3 % oxygen and 5 5% carbon dioxide Riu et al (1966) measured the gas composition by gas chromotography of aspirated air from the middle ear after puncture of the tympanic membrane The mean values were 9 5 % oxygen and 5 5 % carbon dioxide The leakage of air into the cavity and the lack of knowledge of the pressure in the syringe encountered during similar procedures in the maxillary sinus (Aust & Drettner, 1972) was not discussed The material published by Riu et al (1966) consisted of altogether 16 persons, of whom 4 had impaired function of the Eustachian tube

Very few studies of the *gas tension in middle ears with impaired function of the Eustachian tube* have been found The 4 patients with impaired tubal function in the material of Riu et al (1966) had a mean oxygen content of 11 2 % oxygen and differed thus not in the expected direction from the whole material Only one of the patients had middle ear fluid with values of 8 99 % oxygen and 6 15 % carbon dioxide Ingelstedt et al (1967) performed an investigation, not yet published, in which transudate from the air cell system was aspirated and analysed from 7 patients with middle ear transudate, three of whom had malignant tumours in the nasopharynx or pharynx The punctures of the mastoid (mastoideocenteses) were performed under local anaesthesia Mean values of 5 5 % (ranges 4 0 % to 7 4 %) for oxygen and 8 2 % (ranges 7 2 % to 9 5 %) for carbon dioxide were found

Table I *The material and the results of measurements of pO₂ in the transudate of the middle ears and of the function of Eustachian tube investigated by the aspiration–deflation method and by Politzer*

Case	Age	Diagnosis	Ear	pO_2 of middle ear trans (mmHg)	Eustachian tube Aspiration–Deflation	Politzer
1	74	Ca epipharyngis	Left	16	Not investigated	
2	80	Sarcoma tonsillae	Left	88	Not investigated	
3	56	Blue drum since 2 years	Right	10	Blocked	–
4	46	Otosalp chron	Left	40	Blocked	–
5	19	Otosalp chron	Left	10	Not investigated	
6	58	Otosalp chron	Right	43	Not investigated	
7a	72	Ca epipharyngis	Right	45	Not investigated	
7b	72	Ca epipharyngis	Left	68	Not investigated	
8[a]	60	Ca epipharyngis	Right	105	Not investigated	
9	59	Otosalp chron Asthma bronchiale Polyp nasi	Right	42	Blocked	–
10	47	Blue drum since 7 years	Right	108	Blocked	–
11	65	Glioma cerebri Otosalp ac	Left	16	Not investigated	
12	33	Otosalp chron	Right	77	Blocked	–
13	39	Otosalp chron	Left	42	Blocked	–
14	79	Ca epipharyngis Blue drum	Right	26	Blocked	–
15	59	Ca epipharyngis	Right	21	Blocked	–
16	62	Ameloblastoma maxillae	Left	25	Blocked	–
17	75	Ca maxillae	Left	49	Blocked	

[a] Nasopharyngeal cryosurgery under general anaesthesia the day before the investigation

The requirement of avoiding general anaesthesia during such measurements is evident by the investigations performed by Rasmussen (1967), who found that inhaled gases used during general anaesthesia are rapidly transmitted to the middle ear

The purpose of this investigation was to develop a new method for the measurement of pO_2 in the middle ear and present results of such measurements from a series of patients with middle ear transudate

MATERIAL

The material consisted of 17 patients in the ages 19–80 years old One patient was measured in both ears and the rest in one The pO_2 of the transudate was measured in connection with the application of a transmyringeal tube, according to the principles given by Armstrong (1954)

The diagnoses given in Table I show that there were 9 patients with tumours, principally nasopharyngeal carcinoma, and the majority of the rest had chronic otosalpingitis Three had blue drums of long duration, one of them after an operation for a meningeoma

The investigation was performed during the years 1970–73

METHODS

The pO_2 in the transudate was measured within the middle ear and the method did not therefore require aspiration from the middle ear

The measurement was performed on an operation table and a binocular microscope was used Local anaesthesia was given by injection of 1 % Xylocain with exadrine® (Astra) in the skin of the external part of the auditory canal Any anaesthesia accidentally flowing into the auditory canal was sucked away immediately The tympanic membrane was punctured with a cannula fitted around a small pO_2-electrode The tip of the electrode reached immediately behind the end hole of the cannula The electrode, which

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BONE RESORPTION IN CHRONIC OTITIS MEDIA

A Light-microscopical and Histochemical Investigation of Acid Phosphatase Activity

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Abstract As a continuation of our previous work, where we have demonstrated that in chronic otitis media the picture in the submucosa-bone marginal zone is dominated by capillary proliferation and occurrence of a mononuclear, histiocyte like cell containing lysosome like cytoplasmatic bodies, we now report the presence of considerable activity of acid phosphatase in close relation to the eroded bone The activity was localized both extracellularly, spread along the bony surface, as well as intracellularly in mononuclear, histiocyte like cells The acid phosphatase is the "marker" enzyme for lysosomes, and cells with these lysosomes guarantee the presence of enzymatic activity capable of attacking bone collagen It is difficult to avoid the conclusion that the lysosomes and their enzymes are directly involved in the of bone resorption

1972 Thomsen demonstrated that 92% of dislocated incudes after cranial trauma were unharmed, though deprived of their essential blood supply This observation questioned the role of anoxia in the pathogenesis of bone resorption in the middle ear Hentzer & Balslev Jorgensen described in 1972 the submucosa in the middle ear in patients with suppurative chronic otitis, and found the picture to be dominated by cellular and capillary proliferation Thomsen et al (1974*a* and *b*) demonstrated in an ultrastructural investigation of the submucosa-bone border zone in chronic otitis that the overall picture was capillary proliferation and occurrence of mononuclear cells, which we called histiocytes We furthermore demonstrated that there were no significant differences in this border zone with regard to the number and appearance of the capillaries and the histiocytes in chronic otitis media, whether cholesteatoma was present or not Furthermore, remarkably few neutrophilic leucocytes were encountered These observations very much question the role of the cholesteatoma itself in the pathogenesis of bone resorption in chronic otitis, and also point out the improbability of the pressure-anoxia theory, so repeatedly cited in the literature We therefore postulated that these mononuclear cells, with their content of lysosomes and their hydrolytic enzymes (DeDuve, 1959) could play a significant role in the pathogenesis of bone resorption in the middle ear

In order to substantiate this theory we must therefore demonstrate the presence of lysosomal activity in the submucosa-bone border zone Among the hydrolytic enzymes in the lysosomes the acid phosphatase is the most consistent It has been called the "marker" enzyme for the lysosomes

In this paper, therefore, we present the results of light-microscopic histochemical investigations of the submucosa-bone marginal zone in biopsies from the middle ear in patients with chronic, cholesteatomatous otitis media, with special focus on the acid phosphatase In a forthcoming paper we shall report the results of the ultrastructural findings with regard to acid phosphatase and the histiocytes

MATERIAL AND METHODS

Biopsies were taken during middle ear surgery The material consists of 36 biopsies from 10 patients with suppurative chronic otitis media



Fig 1 Light-microscopic photograph of the submucosa-bone marginal zone in biopsy from the middle ear, in a patient with chronic, cholesteatomatous otitis media The dye deposit is due to acid phosphatase activity, spread along the entire eroded surface of the bone (×80)

In each biopsy we attempted to include the cholesteatoma membrane and the underlying submucosa and bone The whole block was gently removed with a fine chisel The biopsies were taken from different parts of the middle ear, epitympanon and mastoid—wherever bone destruction was evident Twenty-four of the biopsies were fixed in cold (4°C) formol–calcium, (4% formaldehyde containing 1% calcium chloride adjusted to pH 7 2 with sodium hydroxide Holt, 1959, Holt et al, 1960) Decalcification was obtained with EDTA for 2–8 days, with storage at 4°C, depending upon the size of the biopsy The specimens were then stored at 4°C in gum arabic (1% gum acacia in 0 88 M sucrose, Holt, 1959, Holt et al, 1960) Just before sectioning, the specimens were frozen in isopentane in a freezing mixture of acetone and solid carbon dioxide (−80°C)

After mounting in the microtome, six sections were made from each specimen, the thickness alternating from 8–10 μm to 50 μm Two of the thin sections (8–10 μm) were stained according to Barka & Anderson's method (1963) for demonstrating acid phosphatase using α-naphthyl phosphatase as substrate and pararosanilin as indicator, at pH 6 5 and room temperature Staining times were 20 min and 30 min The third section was used as control of the staining specificity, either by avoiding the substrate or by applying sodium fluoride as an enzyme inhibitor The specimens were mounted on glass slides and covered with a cover slip

Some of the remaining 12 biopsies were rather large, and could be divided into a total of 22 specimens In 12 of these, 5% glutaraldehyde, buffered with sodium cacodylate 30 mmol/l at pH 7 4, was used as fixative, for 12–24 hours, but otherwise prepared as described above The remaining 10 specimens were fixed in formol–calcium for various periods of time, from 36 hours to 10 days, in order to investigate the influence of fixation time upon the enzyme activity

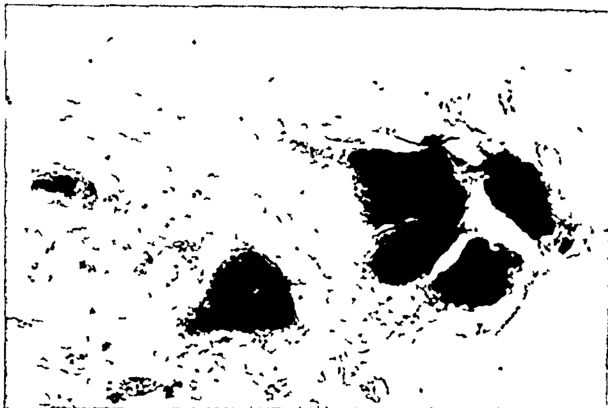
Ten biopsies from 10 patients with otosclerosis served as normal material These biopsies were taken from the promontory or the deepest part of the bony ear canal, where no macroscopic otosclerotic foci were present, and prepared as described above, with 12 hours' fixation in formol–calcium, 48 hours' decalcification in EDTA and stored in cold gum arabic sucrose

As a control of the staining properties of the dye, a specimen of either mouse kidney or lung was stained the same day with the same solution of the pararosanilin as used for demonstration of the acid phosphatase activity in our specimens

RESULTS

All the 24 biopsies, containing submucosa and macroscopically eroded bone from the middle ear in patients with chronic suppurative otitis, fixed in formol–calcium 12–24 hours, decalcified with EDTA and stored in gum arabic, showed a marked acid phosphatase activity in the submucosa–bone marginal zone

The activity with the Barka & Anderson (1962, 1963) simultaneous coupling azo dye method, using α naphthyl phosphate as substrate and pararosanilin as the coupler, is



Fg 2 The submucosa bone marg nal zone in chron c ot t s med a The ac d phosphatase act v ty is represented by the redd sh hexaxon um pararosan l n in th s photo graph as a homogeneous depos t n a local zed eros on of the bone (×400)

icated with a red or redd sh brown dye deposit

The dye was local zed in some specimens as a rather homogeneous deposit spread along the entire surface of the bone (Fig 1) In other parts it was seen in isolated erosions of the bone (Fig 2) in relation to the prol ferated cap llaries here too as a rather homogeneous deposit Furthermore it was clear that some rather large cells (Fig 3) either spread along the bony sur face or at some distance from the bone d splayed acid phosphatase activity This activity was not uniformly spread in the cytoplasm but was concentrated in small particles The cells had only one nucleus and this nucleus was not stained These cells could very well be histio cytes

The 12 specimens fixed in glutaraldehyde for 12 24 hours and decalcified with EDTA showed little or no acid phosphatase activity The specimens fixed with formol calcium for 36 hours or more also exhibited a lack of acid phosphatase activity None of the *controls* of the staining specificity displayed any acid phos phatase activity

On examining the *nori ial* material it was demonstrated that none of these biops es showed any activity either in the submucosa or in the bone when using the fixation decalc fication and staining method described above

DISCUSSION

As stated by Jensen (1973) any kind of fixation of the tissues to be examined by histochemical methods tends to reduce the activity of the enzymes so that ideally unfixed tissue should be chosen for histochemistry However there is a possibility that diffusion of the enzymes in the unfixed tissues might result in an inaccurate



Fig 3 The submucosa bone marginal zone in chronic otitis media stained with the Barka & Anderson histochemical method The phosphatase activity is seen intracellularly in rather large mononuclear cells spread along the eroded surface of the bone (×400)

or missing reaction product The method of fixation in enzyme histochemistry is therefore a compromise

Since it was our main intention to perform electron microscopical cytochemical studies on the *same* specimens as used for the light micro scopical studies presented in this paper we were obliged to fix our specimens

Seligman et al (1951) introduced the use of formalin fixation at low temperatures They demonstrated that fixation for 1 hour at 37°C reduced the activity of acid phosphatase by 59 % whereas the reduction after 2 hours fixation at 4 C amounted to only 21 % The significance of the low temperature has been confirmed by Novikoff (1952) and Holt et al (1960)

The specimens were stored in gum arabic sucrose because of the observations of Krus et al (1961) and Holt (1959) which state that colloid solutions had a protective influence upon the enzymes As far as fixation time is concerned it was evident that fixation in formol calcium for more than 36 hours caused cesation of the enzymatic activity This was to be expected in consequence of the findings of Seligman et al (1951) Novikoff (1952) and Holt et al (1960)

In our laboratory we have generally used 5 % glutaraldehyde as fixative for electron micro scopical examination In previous histochemical examinations of bone we have fixed the speci mens for only 30 min to 2 hours In order to examine the influence of time we fixed 12 specimens for 12–24 hours in 5 % glutaraldehyde These specimens exhibited only little or no acid phosphatase activity

As mentioned above it was still our ultimate goal to demonstrate acid phosphatase activity ultrastructurally and in the *same* specimens as used in this study for light micr opical

demonstration Both fixation and decalcification were therefore unavoidable Even after relatively long periods of EDTA decalcification (up to 8 days), we could still demonstrate considerable amounts of acid phosphatase activity in the specimens As stated by Scarpelli & Pearse (1958), it even seems that EDTA may have a protective influence upon the acid hydrolases

Gomori (1939, 1941) was the first to demonstrate acid phosphatase activity by a histochemical method For electron-microscopical demonstration this is still a most valuable method, but it is well established that for light microscopical demonstration of acid phosphatase, the Barka & Anderson method (1963), using hexazonium pararosanilin as a coupling agent, is the most reliable Pararosanilin was first suggested as coupler by Davis & Ornstein in 1959 Diazotization of pararosanilin results in the formation of hexazonium pararosanilin Although this compound will couple with α naphthyl at pH 5-5 5 (the pH optimum of the acid phosphatase), the coupling rate in this pH range is less than the hydrolysis rate of the [illegible] To avoid diffusion, incubation is [illegible]ed out at pH 6 5, where the coupling rate [illegible] more rapid The compromise in enzyme activity incurred by this non optimal pH is offset by the significant improvement in localization (Barka & Anderson, 1963) The final reaction product is resistant to most organic solvents It is amorphous or microcrystalline, and its colour and form do not change in mounted preparations

The first report of enzymatic activity in chronic otitis media was published by Harris in 1962 He found the acid phosphatase to have a distinct localization in the stratum granulosum of the cholesteatoma epidermis, but no activity was found in the deeper layers of the epidermis and throughout all the subepithelial connective tissues No reaction was found in bone Paparella & Dito (1964) examined the enzyme content of serous middle ear effusions The normal middle ear mucosa was examined histochemically by Lim & Hussl (1969) and Hiraide & Paparella (1971, 1972) Maeda et al (1967) and Palva et al (1970) also localized the acid phosphatase activity to the stratum granulosum, but did not examine the submucosa-bone area Furthermore Palva et al (1970) have investigated the lactate dehydrogenase pattern of the middle ear mucosa in chronic otitis with cholesteatoma as well as the esterases (Palva et al, 1971)

Abramson (1969) and Abramson & Gross (1971) have studied the activity of collagenase in middle ear cholesteatoma They found the highest collagenolytic activity when granulation tissue was present, and that granulation tissue in itself could lyse the collagen

In contrast to the finding of Harris (1962), Zechner et al (1968) demonstrated that highly positive acid phosphatase activity was visible when studying bone trabeculae obtained at tympanotomy The strongest ferment activity was noticeable in the granulation tissue lying closest to the bone Until now, this observation has remained unnoticed

In order to discuss bone resorption in general, one must have in mind the composition of bone According to Eastoe (1956), bone consists of inorganic material, mostly calcium phosphatate, collagen, water, about 20 %, mucopolysaccharide and resistant protein

Under resorptive stimulus the bone cells produce considerable amounts of acids, mostly lactic and to a lesser extent citric acid, due to glycolytic activity (Borle et al, 1960, Nichols, 1963) The lowering of the pH makes the calcium phosphate salts of the mineral easily soluble

There are two possible mechanisms for the removal of the decalcified collagen matrix degradation by a specific collagenase active, at neutral pH, of the native collagen molecule, or digestion by non specific, possibly lysosomal, acid hydrolases after denaturation of the molecule by local accumulations of metabolic acid

Collagenolytic activity at neutral pH has been shown to exist in isolated bone cells from animals and man (Walker et al, 1964 Woods & Nichols, 1965, Abramson, 1969 and Abramson & Gross, 971)

Likewise, Vaes (1969) has shown that bone cells contain lysosomes, and that the rate of synthesis and release of typical acid hydrolases are markedly increased by parathyroid hormone in tissue culture Thus both mechanisms for collagen breakdown seem to exist in bone Vaes (1964) and Vaes & Jacques (1965) have furthermore demonstrated that lysosomal hydrolytic enzymes are capable of degrading mucopolysaccharides The same lysosomal enzyme would presumably be able to remove bone cells But which cells are responsible for the bone resorption? It is generally accepted that the multinuclear osteoclast, with its lysosomal hydrolases, is capable and responsible for the normal resorption (Gonzales & Karnovsky, 1961, Hancox & Boothroyd, 1963, Young, 1963, Irving & Heeley, 1970) Several authors have presented evidence suggesting that the osteocytes are capable of resorbing their lacunar walls (Jaffe, 1933, Heller Steinberg, 1951, Belanger et al, 1963, Belanger, 1965, Belanger et al, 1965, Frost et al, 1960, Belanger, 1969, Nichols, 1970) Jaffe (1933) and Cameron (1961) have indicated that endothelial cells may be implicated in resorption Muhlethaler (1953), Goldhaber (1961), Andersen & Mathiessen (1966), Chevance et al (1970) and Bretlau et al (1971) have suggested that histiocytes are capable of bone resorption, or they can serve as precursors of osteoclasts (Hancox, 1956, Jee & Nolan, 1962)

Conceivably, these cells represent mesenchymal specializations which have retained their capacity to resorb and are capable of doing this under suitable circumstances (Young, 1963) Goldhaber (1961, 1963) demonstrated that high oxygen tensions induced an osteoclastic type of bone resorption with increased collagenolysis (Stern et al, 1966) Sledge (1965) and Sledge & Dingle (1965) could release and activate the lysosomes by hyperoxia in cartilage resorption Allison (1965) could also activate the lysosomes of macrophages by excess oxygen It is therefore conceivable that increased oxygen tensions could play a significant role in the release of hydrolytic, lysosomal enzymes from the mononuclear, histiocyte like cell with subsequent bone resorption in chronic otitis media

Apart from high oxygen tensions, other factors may have an influence on bone resorption Stern (1971) could induce resorption of living bone with the presence of serum albumin, and the effect was concentration dependent Hausmann et al (1972) could stimulate bone resorption with highly purified lipopolysaccharides, and Hausmann et al (1973) increased resorption in tissue culture by adding unheated normal serum Puche et al (1973) concluded that insulin stimulates bone resorption

With regard to the role of cholesteatoma in bone resorption in patients with chronic otitis media, we believe we have demonstrated that the cholesteatoma itself is not necessary for bone resorption (Thomsen et al, 1974*a* and *b*) We have furthermore demonstrated that the cellular population of the submucosa-bone marginal zone is essentially the same whether cholesteatoma is present or not We have demonstrated that capillary proliferation is a cardinal finding in the marginal zone, thus stressing the improbability of the anoxia theory in middle ear bone resorption The dominating cell in the eroded marginal zone is a mononuclear, histiocyte like cell, with dense cytoplasmatic bodies, of lysosomatic character

In this paper we have, in the light microscope, shown that the marker enzyme for lysosomal activity, the acid phosphatase, is present in large quantities along the eroded bone in the middle ear, both extracellularly as well as intracellularly in mononuclear cells As stated by Vaes (1969) it appears difficult to avoid the conclusion that the lysosomes and their enzymes are directly involved in the processes of bone resorption However, in chronic otitis media, the enzymatic activity seems to originate from a histiocyte-like cell, rather than from a multinucleated, giant-cell, osteoclast, normally found in resorptive zones We have not been able to demonstrate any of these giant-cells This finding corresponds to the observations of Chevance et al (1970) and Bretlau et al (1971) that on the active, otosclerotic front the could

never find a single osteoclast, but the picture was dominated by mononuclear, histiocyte-like cells, showing acid phosphatase activity in the lysosomal-like bodies in the cytoplasm

This paper has not shed any light on the origin of the cholesteatoma itself But our examinations show that the bone resorption in chronic, cholesteatomatous otitis media can be dependent on the enzymatic activity of a mononuclear, histiocyte-like cell, located close to the bone The presence of acid phosphatase activity indicates the presence of lysosomes, and cells with these lysosomes guarantee the presence of enzymes capable of attacking bone collagen

In a forthcoming paper we will try to demonstrate the ultrastructural localization of the acid phosphatase in relation to bone destruction

ZUSAMMENFASSUNG

Wir haben in fruheren Mitteilungen dargestellt, dass das Aussehen des Grenzgebiets des Submucosa Knochens in chronischer Otitis media von kapilarer Wucherung beherrscht ist sowie von der Entstehung mononuklearer, histiozyt-ahnlicher Zellen, die lysosom ahnliche zytoplasmatische Korper enthalten Im Anschluss daran zeigen wir nunmehr, dass eine bedeutende Aktivität von aurer Phosphatase in enger Nachbarschaft des erodier Knochens stattfindet Diese Aktivitat wurde sowohl zellular — uber die Knochenoberflache zerstreut lokalisiert als auch intra zellular — in mononuklearen tiozyt ahnlichen Zellen Die saure Phosphatase ist ein Indikatorenzym fur Lysosome, und demzufolge beweist das Vorhandensein von Zellen mit Lysosome Inhalt das Vorhandensein derartiger enzymatischer Aktivität die Knochen Kollagen angreifen kann Man kommt kaum umhin die Schlussfolgerung zu ziehen, dass die Lysosome und ihre Enzyme in der Knochenresorption direkt involviert sind

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DIRECTIONAL PREPONDERANCE 1942–1974

A Review

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Abstract The subject reviewed is the phenomenon of Directional Preponderance (DP) a term first proposed in 1942 by Fitzgerald & Hallpike for an abnormality in the pattern of the caloric responses found to occur in association with a variety of unila eral lesions, both central and peripheral of the vestibular system On the basis of observations carried out upon a series of human subjects, Fitzgerald Hallpike & Cawthorne (1942) argued that the DP towards the unaffected labyrinth which follows unilateral labyrinthectomy is subserved in part by a unilateral loss of utricular function —a utricular paresis Support for the utricular paresis hypothesis as developed in the course of subsequent studies by Hallpike and his co-workers is thought to be provided by a substantial body of evidence both clinical and experimental which has since become available This is reviewed

The primary purpose of the Caloric Test Procedure as described by Fitzgerald & Hallpike (1942), was diagnostic A measured stimulus was applied to and evoked a nystagmic response from a known sense organ, the external canal This provided a measure of its reactivity However, mixed up with and distorting the response, another factor was sometimes present—Directional Preponderance, or DP as it is now termed This might occur with organic damage at all levels of the nervous system, from the cerebral cortex to the labyrinth

In some cases it had a useful localizing value In others, this was less evident So much so, that for many who look to the caloric tests for help with their diagnostic problems, DP would since seem to have become a source of disappointment and confusion The situation is certainly not one that can be welcomed and a survey of the literature in the course of the last 30 years reveals as its likely cause a number of serious theoretical uncertainties

Is it possible, for instance, to maintain that the DP which follows a unilateral hemispherectomy is attributable to the functional elimination of any particular part of the hemisphere? If so, what is the neurological mechanism which relates it to the DP? Again, in the case of DP found in association with lesions of the labyrinth and brain stem, to what extent can this be accepted as evidence of the involvement of utricular mechanisms?

In the course of the last few years, new evidence on these issues has become available, and will be considered

For certain basic ideas it is still convenient to refer to the Caloric Response diagrams introduced by Fitzgerald & Hallpike (1942)

In Fig 1 are shown for an average normal subject the 4 Caloric Response durations,[1] left and right, for the cold and hot stimuli The interrupted lines 1 and 4 represent nystagmus to the right, lines 2 and 3, nystagmus to the left The effect upon the pattern of the Caloric Responses of Directional Preponderance is shown in Fig 2 With DP to the right, 1 and 4 are increased with respect to 2 and 3 With DP to the left, this relationship is reversed

In Fig 3 is shown another abnormality of the response pattern For the most part it is

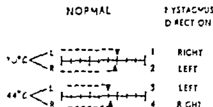


Fig 1 Caloric test results Average normal subject

encountered in association with organic affections involving the sensory end organ of the horizontal canal and/or its nerve fibres The result, as shown, is a reduction of caloric reactivity on the one side or the other For this, the term 'canal paresis' was proposed by Fitzgerald & Hallpike and continues to give good service

Canal paresis may be associated with a DP The caloric response pattern is then a mixed one Nevertheless, in it, these two components remain easily distinguishable

In Fig 4 is shown such a combination There is a right canal paresis (1), also a DP to the left (2) If the right canal paresis is introduced into pattern 2 the resultant will be as shown at (3), with the two cold responses about equal, and the two hot responses widely separated This exaggeration of the hot response on the unaffected side is a very striking clinical feature

It will be noted that, for various reasons, the magnitude of the responses was measured in terms of their duration Nowadays, regrettably, this parameter is out of fashion Instead, it is widely claimed that *nystagmus magnitude* should always be expressed in terms of slow component speed, a requirement that calls for an electronystagmograph with circuitry as prescribed by Henriksson (1955) There is, of course, nothing wrong with this All the same, it seems worth recalling that Lorente de Nó, who contributed so much to the scientific foundations of clinical nystagmology, relied very much *upon duration measurements and said of the slow* component speed that, by and large, it goes hand in hand with duration In other words, the stronger reactions—stronger, that is, in terms of their slow component speed—are also the longer Though not strictly correct, this statement is still quite close to the mark and there are certainly good reasons why clinicians who use the caloric tests should continue to measure nystagmus durations While doing so, there is no reason either why due account should not also be taken of slow component speed This manifests itself in a compound of frequency and amplitude that may be *called* 'briskness" Of it, a useful measure can be derived from direct observation and can be incorporated in the response diagram as illustrated in Fig 5 This shows a very slight Reduction of Responses, 2 and 4, i e, a very slight Right Canal Paresis In terms of duration the reduction is negligible However, when taken in conjunction with an obvious difference in 'briskness' which was observed and is simply indicated in the diagram, it can be accepted as significant It is easy in this way to include in the diagram quite a useful comparison of the slow component speeds of the four responses

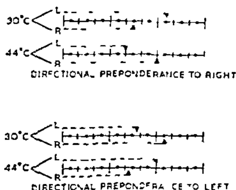


Fig 2 Caloric test results. Directional Preponderance

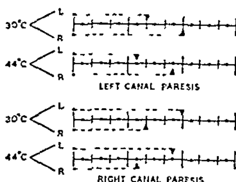


Fig 3 Caloric test results Canal paresis.

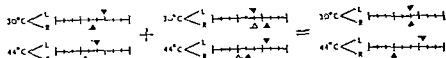


Fig 4 Caloric test results. Combined pattern Right canal paresis with Directional Preponderance to the left

Another point of great importance concerns the extent to which the magnitude of evoked nystagmus depends upon eye positioning Here, of course, something can apparently be seen of the working of Alexander's Law

When the caloric tests are carried out according to the procedure prescribed by Fitzgerald & Hallpike, the eye positioning is known, and is maintained This adds greatly to the constancy of the results If, however, the tests are carried out with the eyes closed, eye positioning is much more difficult to control and even to specify In consequence, the nystagmic responses, as recorded nystagmographically, though larger, are more difficult to evaluate and may be seriously misleading

PART I

DP of Induced Nystagmus resulting from Cerebral Lesions

As the first object of their attention, Fitzgerald & Hallpike selected the DP known to occur in *association with unilateral cerebral lesions* De Kleyn and others had shown that in rabbits following the removal of one hemisphere, there was a well marked DP of induced nystagmus to the side of the lesion Fitzgerald & Hallpike (1942) investigated the matter in a group of human subjects with unilateral cerebral lesions The lesions were localised to various parts of the hemisphere, and DP, always directed to the side of the lesion, was found to occur if it involved the posterior part of the temporal lobe The DP was usually quite a small effect and this the authors were careful to emphasize (Cawthorne, Fitzgerald & Hallpike, 1942)

Clinical and experimental support for these findings has been substantial Thus, Andersen and his colleagues at Aarhus (1954) using the same caloric test procedure in a series of subjects with unilateral cerebral lesions, were able to confirm the findings More recently (1965), it was shown by Arslan & Molinari that electrical stimulation of the posterior part of the temporal lobe in the guinea pig has a marked effect in modulating the resting discharge in certain of the vestibular nuclear elements in just the manner postulated by Fitzgerald & Hallpike Still more recently (1970) Minnigerode *demonstrated that cooling of the* posterior part of the temporal lobe in the guinea pig causes ipsilateral nystagmus

However, conflicting claims have also been made For instance, Hakas & Kornhuber working in Jung's Clinic described in 1959 the results of similar studies in human subjects with unilateral cerebral lesions These showed that with lesions of the posterior temporal area, such DP of induced nystagmus as could be demonstrated was directed, not to the side of the lesion, but to the other However, the test procedure used differed from that prescribed by Fitzgerald &

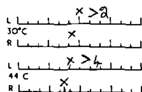


Fig 5 Caloric test results Right canal paresis The reduction of the right responses very slight in terms of duration is marked in terms of briskness

Hallpike in one very important particular—visual fixation being discarded and the nystagmus recorded electrically with closed eyes That the findings might have been vitiated by uncontrolled conjugate deviations of the eyes, or by latent tendencies thereto, was an obvious possibility and of this Carmichael, Dix, Hallpike & Hood (1961) qere soon able to adduce very strong evidence

They used a series of subjects with unilateral cerebral lesions, all of whom showed DP of caloric and rotational nystagmus towards the affected hemisphere, i e when the tests were carried out with the eyes open and with visual fixation maintained in the straight ahead line When the tests were repeated with the eyes closed, the results were quite different, and in many the DP was now towards the unaffected hemisphere An additional observation recorded by these workers was that in nearly all of their subjects there occurred a conjugate deviation of the eyes Its direction coincided with that of the DP and it must in all probability have been a factor in determining it It was considered be of considerable significance

Of the findings of Hakas & Kornhuber (1959), ems reasonable to say that they illustrate the nfusion that is likely to arise if nystagmic actions are evaluated without proper regard to eye positioning

As to the phenomenon itself, there would seem to be little ground, on the balance of the evidence, for disputing its occurrence as Fitzgerald & Hallpike reported it, assuming again a strict adherence to the test procedure which they specified

Certainly, the phenomenon may at times be a small one, yet on this point too, emphasis had already been laid (Cawthorne Fitzgerald & Hallpike, 1942) It should still retain a considerable measure of diagnostic significance

There remains the problem of its mechanism Here, a number of questions arise How, for instance, is it related, as in some ways it must be, to that of the DP that so commonly results from lesions at lower levels of the vestibular system, including the labyrinths?

Fitzgerald & Hallpike supposed that the posterior temporal lobe areas exerted some balanced tonus effects upon the vestibular nuclei A lesion of one of these areas would put these tonus effects out of balance and this would cause the DP of induced nystagmus which they reported More, obviously, would need to be known of the mechanism of such a tonus effect—whether, for instance, it involves and operates through particular elements of the vestibular nuclei which are specifically associated with the nystagmic mechanism Of this, no evidence could be adduced and a later paper (1965) Carmichael, Dix & Hallpike developed another line of thought, namely that the Directional Disturbance (DP) of induced nystagmus in question arose from the derangement of certain ophthalmostatic mechanisms of which Bartels (1912) and others have written and which have to do with the tonic control of eye positioning Here, the neurological mechanisms concerned show some relevance to those which underlie the operation of Alexander's Law, and, as argued by the authors and also by Hallpike in other publications (1965, 1967), provide useful guide lines for the study of the other and common variety of DP which forms the subject of Part II of this paper

PART II

DP of Induced Nystagmus occurring at the Result of Unilateral Lesions of the Labyrinth or of the Peripheral Vestibular Neurones

Destruction of one labyrinth—let it be the left—causes spontaneous nystagmus to the right This abates rapidly, and usually disappears within a few weeks The caloric responses at this stage show a typical variant of the mixed pattern, to which earlier reference has been made

This is illustrated in Fig 6 The patient was a middle aged woman with a history of paroxysmal vertigo with deafness and tinnitus of the left ear She was treated by means of a surgical destruction of the left labyrinth Before the

operation the caloric responses showed only a slight DP to the right Following the operation the left responses were, as shown, abolished The responses of the unaffected labyrinth showed a typical distortion, with exaggeration of the hot response—a severe DP to the right

In 1942 Fitzgerald & Hallpike examined this situation, seeking an explanation of the nystagmus itself, sustained as it might be, for a week or more after the operation, also for its subsequent prolongation in the form of a DP of the caloric responses of the contralateral labyrinth For this investigation (Cawthorne, Fitzgerald & Hallpike, 1942) they were fortunate in having for their material a number of subjects upon whom the late Sir Terence Cawthorne had carried out destruction of the labyrinth for unilateral Meniere's Disease They argued, as others had done, that the nystagmus was due to some unbalanced tonus effect emanating from the unaffected labyrinth What, they asked, was the actual sense organ or organs involved?

In 1936 Lowenstein & Sand had demonstrated the occurrence in the horizontal canal nerve of the dog-fish of a resting action potential discharge, waxing and waning in response to ampullo-petal and ampullo-fugal displacements of the endolymph This, in itself, meant, that at any rate in this species, the canal itself was such a tonus organ

However, Ross (1936), working at Cambridge on the frog, did not confirm this His general conclusion was that in the absence of movement or vibration, the labyrinthine receptors were electrically inactive

This led Fitzgerald & Hallpike to look elsewhere for their tonus source The experiments of Magnus (1924) and his co-workers had already thrown much light upon the tonus action of the labyrinthine sense organs in the control of mammalian posture Later, their findings were more closely analysed, and in some ways extended, by Tait & McNally (1925), who used a micro-dissection technique to section, in the frog, the individual nerves from these organs Their results were highly significant Thus,

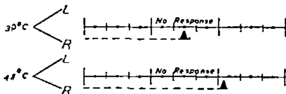


Fig 6 Caloric test results 7 weeks after left labyrinthectomy

while section of the nerves from the canal organs or saccule had no apparent effect upon posture, section of the nerve from the utricle caused disturbances which were severe and lasting

If, therefore, as seemd to be the case, the utricle was the dominant source of labyrinth tonus, it seemed reasonable to Fitzgerald & Hallpike to accept it as the tonus organ responsible for the sustaining of the nystagmus which followed unilateral labyrinth destruction in the mammal They accordingly allotted to it an essential part in the neural mechanism of this nystagmus Since, at the time, there seemed to be some lack of evidence that the mammalian semicircular canal itself possessed any tonus function, they were led to leave this organ out of their reckoning Reasonable as this seemed at the time, fresh evidence soon showed it to be unjustified

Not long after Ledoux (1958), began his systematic investigations of the action-potentials in the nerve branches of the frog's labyrinth, and soon showed that the situation in the canal nerves in this species was just as Lōwenstein had described in the dog fish In the horizontal canal nerve, for instance, there was the same resting or tonic discharge, waxing and waning with ampullo petal and ampullo-fugal displacements of the endolymph In the light of Ledoux's work, it might at the time have been deemed appropriate to say this of the nystagmus which follows a unilateral labyrinth destruction that it is a tonus effect emanating from the nystagmic mechanism of the contralateral, intact canal This soon undergoes central compensation and disappears As it does so, it will continue to manifest itself in the characteristic mixed

DIRECTIONAL PREPONDERANCE	21 CASES
CANAL PARESIS	49 CASES
DIRECTIONAL PREPONDERANCE + CANAL PARESIS	18 CASES
NORMAL	12 CASES

Fig 7 Caloric test results in 100 cases of Meniere's disease

pattern of the caloric responses Finally, this residuum also disappears, and there is left only a complete canal paresis

According to Ledoux (1973), he has seen this sequence enacted in not a few subjects with vertigo due to organic affections of the peripheral vestibular apparatus—Meniere's Disease and Vestibular Neuronitis In these, the caloric tests were carried out soon after the onset of the vertigo, and subsequently repeated over a period of years Ledoux would therefore argue that to explain these findings it is necessary only to accept his evidence that the canal has a tonus function The utricle would not then need to be brought into the matter at all This point of view is by no means an isolated one and neither McNally (McNally & Stuart, 1967) nor Jongkees 969) in their reviews of the field, accord any port to the possibility of a utricular par-cipation in the nystagmic mechanism as proposed by Fitzgerald & Hallpike

There can be no reason to doubt the correctness of Ledoux's clinical observations The sequence which he describes does occur, and indeed, as Hallpike has clearly pointed out (Cawthorne & Hallpike, 1943), DP, manifesting itself as a mixed pattern of the caloric responses, may indeed be observed in the *course of a resolving spontaneous nystagmus*, resulting from certain vestibular lesions In this context, at any rate, it is certainly reasonable to accept Ledoux's contention that the DP which he observed could be explained in terms of a canal lesion, that is to say, of a lesion affecting the canal and limited thereto Clearly, we need assign to a utricular lesion no part in this picture

All the same, it needs to be added that the story which it tells is incomplete and misleading The fact is, that other situations occur in which utricular lesions are certainly present and from these, good evidence can be derived that they do indeed play a part in bringing about a DP *of induced nystagmus*

The evidence in question is both clinical and experimental The former will be examined first Here, an item of importance is provided by the earlier studies of the caloric test findings in a group of subjects with Meniere's Disease (Cawthorne & Hallpike, 1943) If in these a DP, when it occurred, were always due to a tonus imbalance resulting from canal damage, it would seem reasonable to expect that it would of necessity be accompanied in all cases by evidence of such damage, in the form of a canal paresis In other words, the caloric response pattern would always take one of two forms Firstly, there might be a canal paresis, let it be a left canal paresis With this, representing the *uncompensated residuum of a canal imbalance* nystagmus, there would be a DP to the right This would take the form of a characteristic exaggeration of the right hot response Alternatively, the compensation might be complete and there would then remain a left canal paresis alone But, as shown in Fig 7, this was not quite what was found 49 of the 100 cases showed CP only A further 18 % showed the combined pattern Clearly, in all the cases in these two groups, the findings could be explained in terms of a labyrinthine lesion limited to the horizontal canal

But this was not all Vogel in 1929, working in Von Eicken's Clinic, had reported that in Meniere's Disease, DP of the caloric responses *might occur alone As shown in Fig 7 this was* found also in 21 % of the cases reported

Combining these two groups—canal paresis and the combined pattern, i e 67 % of all the cases observed—it might be inferred with reason, that in Meniere's Disease the organic damage most commonly affects, and is often limited to, the semicircular canal mechanism Viewed in this light, another point also emerges as judged by their symptons, the durations of



Fig 8 Rabbit, showing typical disturbance of posture resulting from destruction of left labyrinth (Magnus)

the disorder had, in many of the subjects, been short, a matter of months rather than years Nevertheless, spontaneous nystagmus was very rarely observed and its residuum, a combined pattern of the caloric responses, in only 18% of the cases This permits a further inference namely, that the central compensation of a canal imbalance nystagmus is characteristically speedy and complete

There remains, finally, the group in which DP occurs alone Because, in these subjects, there was no evidence that the canal was affected, it was argued that some other organ must be, and that this organ must be the utricle While evidence for this was considered to be substantial, it was admittedly indirect What, *clearly*, *was needed to be known*, *is what* happens in subjects in whom the evidence of a utricular lesion is direct and certain

This provides the substance of a second item of clinical evidence—the ocular disturbances which follow complete unilateral destruction of the mammalian labyrinth, including that of man In this situation more than one imbalance phenomenon is involved Nystagmus, of course, is one This has already been considered and attributed to the unbalanced tonus action of a nystagmogenic mechanism, the horizontal canal of the contralateral and unaffected labyrinth

But there is also another kind of imbalance, something that Bartels (1912), a pupil of Ewald, has aptly termed an ophthalmostatic imbalance The mode of operation of this mechanism in the case of a left labyrinth de struction in the mammal will be briefly considered In Fig 8 is shown a rabbit some 48 hours after this procedure The head has been turned to bring the affected ear to the front of the animal This is what Magnus has described as the *Grunddrehung*, or basic twist, and is due in very large measure and beyond all doubt, to the unbalanced action of the right utricle The eyes will show a nystagmus, with its rapid component to the right, but they will also be held in a position of left deviation *These finding may now be transferred to* a human subject immediately following a left labyrinth destruction, seen as he usually is, in bed He will be supine instead of prone, with his left ear not undermost as shown in Fig 8, but uppermost, a point that has always been stressed since the days of Barany Now as Barany (1907) explained the matter in one of his lectures, this, for the patient, was a position of convenience, meaning that with the right side of the face on the pillow and the eyes deviated to the left, this, being the position of minimum nystagmus, would enable him to look around him

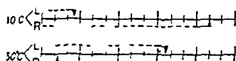


Fig 9 Caloric test results Guinea pig Showing Directional Preponderance to the left following injury to the right utricular nerve

with the minimum of visual disturbance All this, of course, is reasonable enough What Barany *does not seem to have considered* so much is something that becomes clear from the later work of Magnus (1924) and his collaborators

This is that the position of the patient in bed, apart from being one of visual convenience, is really imposed upon him as part of the *Grunddrehung* so clearly seen in the rabbit, and there due, beyond all reasonable doubt, to the unopposed tonus action of the right utricle

If, therefore, it can be accepted that the posture of the head and neck in a human subject is part of this *Grunddrehung*, so too, in all probability, *is that of the eyes Both, thus,* become attributable to the unbalanced action -the unaffected utricle That such an ocular tion should be brought about by the utricle of course, in good accord with certain items experimental evidence For example, Ulrich (1934) and others have repeatedly shown that ocular deviations constantly result from mechanical and electrical stimulation of the utricular macula Thus, it comes as no surprise to find Bartel s ophthalmostatic deviation as one result of a unilateral labyrinth destruction

A number of questions, of course, arise how, *in particular, can this deviational mechanism* exert upon the nystagmogenic mechanism of the canals, the directional effect that is seen in a DP to the unaffected side of caloric nystagmus To this and other matters, later reference will be made First, however, certain items of experimental evidence need to be considered

For the experimental study in man of the action of the otolith organs upon canal-evoked nystagmus, the particular *combinations of* physical stimuli required are obtainable by means of the centrifuge, and the work of Lorente de Nó (1931) in this field is well known More recently, Bergstedt has used the centrifuge for detailed study of human positional nystagmus Some of his findings, presented in a valuable monograph (1961), are relevant to, and in Bergstedt's view, provide basic experimental support for the utricular hypothesis of the DP mechanism

In the field of animal studies, experimental evidence *relating to the function of the* mammalian utricle has for long been scanty That this should be so, arises in large measure *from the difficulties which beset the micro-anatomical* approach to the individual sense organs and nerve branches of the mammalian labyrinth These difficulties are in the course of being overcome Owada and his colleagues in Japan (1960) and more recently Fluur & Siegborn in Stockholm (1973) working respectively upon the guinea pig and cat, have been able to isolate and section selectively the nerves from the horizontal canal and utricle The results which *they report seem to be highly significant*

The anatomy of these nerve branches is easily described They leave the internal auditory meatus at its uppermost and posterior corner, and then pass horizontally backwards lying embedded in the outer bony wall of the vestibule just above the oval window Here, they are covered by the facial nerve as it lies in the Fallopian canal Their exposure in the cat calls for the opening of the middle ear, and the stripping away of the facial nerve The nerves from the horizontal canal and utricular macula can then be separately sectioned

Section of the canal nerve produces contra lateral nystagmus This, of course, would be as expected—a canal imbalance nystagmus In addition, however, Fluur and his colleagues have clearly shown that section of the utricular nerve has the same effect—a contralateral nystagmus This is a remarkable finding and provides what would seem to be a decisive confirmation of the hypothesis advanced by Fitzgerald & Hall *pike that the contralateral nystagmus which*

results from a unilateral labyrinth destruction is subserved in part by a utricular lesion

Owada and his co-workers, using a different approach, carried out similar experiments in the guinea pig and were able to reproduce the situation of so much clinical interest—a patient with a unilateral utricular lesion being submitted to the caloric tests In Fig 9 is shown the pattern of the caloric test responses obtained in one of their animals The Right Utricle Nerve has been injured and the pattern shows a clear DP to the left

As to the mechanism of the utricular contribution to the contralateral nystagmus which follows a lesion of one labyrinth, it seems reasonable to conclude that it is an indirect one Thus, in the case of a left labyrinthine lesion with involvement of the canal and utricle, the nystagmus will primarily be a canal imbalance nystagmus But the Dominant Right Utricle will contribute to this indirectly This it will do by bringing about an ophthalmostatic deviation of the eyes to the left, so supplementing the slow component of the nystagmus to the right

If this can be accepted, it needs next to be asked where in the nervous system the coupling of these nervous mechanisms—canal imbalance and utricular imbalance—takes place Here, the experimental findings of Duensing & Schaefer (1959) are highly relevant They recorded from single cells of the vestibular nucleus in the cat, and obtained clear evidence of the convergence upon these cells of action potentials from the canal and otolith organs It would therefore seem likely that the coupling in question takes place in this area of the nervous system or at some point between it and the sense organs con cerned

ZUSAMMENFASSUNG

Gegenstand der vorgelegten Übersicht ist das Phanomen des, Richtungsuberwiegens (Directional Preponderance, DP) Diese Bezeichnung ist 1942 erstmals von Fitzgerald und Hallpike fur ein pathologisches vestibulares Reizantwortmuster gewahlt worden, das durch thermische Labyrinthreize ausgelost wird und in Verbindung mit zahlreichen einseitigen, peripheren und zentralen vestibulären Funktionsstorungen auftreten kann Ausgehend von Beobachtungen, die an einem umfangreichen klinischen Material gewonnen wurden, haben Fitzgerald, Hallpike und Cawthorne (1942) den Schluss gezogen, dass das Richtungsuberwiegen (DP) zur gesunden Seite das im Anschluss an einen Labyrinthausfall auftritt, zum Teil durch den einseitigen Verlust der utrikularen Funktion gefordert wird (sog utricular-paresis) Diese „utricular paresis' Hypothese, die spater von Hallpike und Mitarbeitern weiter entwickelt worden ist, erhalt durch neuere Ergebnisse klinischer und experimenteller Forschung weitere Unterstützung

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THE FUROSEMID TEST FOR MENIERE'S DISEASE

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Abstract In a previous report it was suggested that intravenous administration of furosemid followed by a comparative caloric test (the furosemid test) could be utilized for detection of endolymphatic hydrops (Kitahara et al, 1973) As a result of the further application of the furosemid test upon 161 patients with vertigo positive results were observed in 80% of patients with typical Meniere's disease 6% of atypical Meniere's disease, 42% with labyrinthine syphilis and 27% with sudden deafness In patients with labyrinthitis results were negative It is thus concluded that the furosemid test provides positive evidence for determining the etiology of vertigo

Meniere's disease is at present diagnosed by clinical symptoms such as episodic vertigo, tinnitus and loss of hearing Clinical and laboratory studies are directed to the elimination of extralabyrinthine disease as well as labyrinthine disease with known etiologies Treatment for Meniere's disease, such as decompression surgery or chemotherapy utilizing diuretics, is designed to directly alleviate endolymphatic hydrops (Portmann, 1927 House, 1962, Shea, 1966, Arslan, 1970, Klockhoff & Lindblom, 1967) The possibility of furosemid administration for the detection of endolymphatic hydrops has previously been discussed by the authors In cases of typical Meniere's disease a diagnosis of endolymphatic hydrops can be clinically made without difficulty when checking characteristic features Patients with endolymphatic hydrops may also have a hidden cochlear symptom or other morbid affections such as syphilis or sudden deafness The few reports of such cases include Opheim (1950), Williams et al (1950), Lindsay & Schulthess (1958), Bosatra & Stefani (1965), Karmody & Schuknecht (1966), Pulec & House (1973) Should the furosemid test reveal the existence of hydrops in the labyrinth of these patients with labyrinthine vertigo, then such would be a good method of choice among medical and surgical treatments The aim of the present investigation was to determine the effectiveness of the test, not only in typical Meniere's disease but also in the other categories of labyrinthine disease

MATERIAL AND METHODS

Among 161 patients with vertigo, 93 had typical Meniere's disease, 34 atypical Menière's disease, 12 labyrinthine syphilis, 11 sudden deafness, and 11 had labyrinthitis There were 23 normal controls Diagnosis of typical Meniere's disease was limited to patients with recurrent attacks of idiopathic vertigo, perception deafness, and tinnitus Recurrent attacks of idiopathic vertigo without these symptoms were regarded as atypical Meniere's disease It is our opinion that the latter is synonymous with Williams' "Meniere's disease without cochlear symptom' or Kornhuber's "Akute isolierte Vestibular Störungen '

The patients with labyrinthine vertigo and a positive Wassermann were included into both congenital and acquired labyrinthine syphilis accompanied by other qualitative examinations in the C S T and TPHA tests Diagnosis of sudden deafness was restricted to the cases in

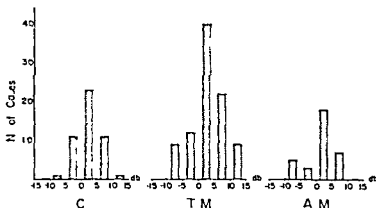


Fig 1 Effect of furosemid on hearing. *C* controls *TM* typical Menière's disease *AM* atypical Menière's disease

which a negative Wassermann patient could state accurately the date of sudden onset of a very severe deafness, following a single attack of vertigo Labyrinthitis was confirmed opera- vertigo with otorrhoea in 2

Patients with extralabyrinthine lesions which could induce the attacks were carefully elimi nated from each category classified above by clinical and laboratory examination

Immediately preceding an i v injection of furosemid 20 mg, pure tone audiometry and the caloric test were performed, and subjective symptoms such as headache or tinnitus were corded The index of hearing response was culated as the average of the hearing thresholds recorded at 250, 500, and 1 000 Hz The caloric test was performed with 50 cc of water at 30 C, and ice-cold water was used in cases where no reaction was obtained with this stimulus DC nystagmography was employed to measure the maximum velocity of caloric nystagmus as the basis of evaluating labyrinthine function These tests were repeated 1 hour after the injection, at which time, in most cases, the diuretic effect had reached its maximum The two sets of data were then compared

RESULTS

Subjective symptoms

In 35 (38%) out of 93 cases of typical Meniere's disease, tinnitus was relieved after administration of furosemid In 4 (33%) out of 12 patients with syphilis, the tinnitus diminished There was no particular effect in the other groups

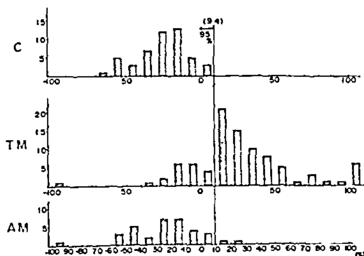


Fig 2 Changes in caloric response following furosemid application Percentage of increase or decrease in maximum velocity is demonstrated in each group Abbreviations as in Fig. 1

Hearing response

In 31 out of 93 cases of typical Meniere's disease, an improvement of hearing over 5 dB was observed However, there were no significant changes in hearing in any of the groups, as shown in Fig 1

Caloric response

Percentages of increase or decrease in maximum velocity of caloric nystagmus in normal subjects, typical Meniere's disease and atypical Menière's disease after the administration of furosemid are shown in Fig 2 According to statistical analysis of the control group, 95 % of normal population falls below +9 4% Therefore, the value of +9 4% was defined as the upper limit of normal range (the 90 % tolerance limits are +9 4 % and −44 6 %) The effect of furosemid on caloric response of diseased ear in the five groups of labyrinthine disease is shown in Table I Increase in maximum velocity of caloric nystagmus beyond the normal range (+9 4 %) is referred to as positive, all others are negative

Positive reactions were evident in 80 % of typical Meniere's disease, 6 % of atypical Meniere s disease, 42 % of labyrinthine syphilis and 27 % of sudden deafness No positive reaction is obtained in cases of labyrinthitis

Side effects

Except for one patient with atypical Meniere's disease who complained of a slight headache, side effects were nil

DISCUSSION

Furosemid, a derivative of anthlanilic acid, which was synthesized by Muschaweck & Hajdu in 1964, induces a rapid diuresis by depressing the reabsorption of water, sodium and chloride in Henle s tubule The increase in caloric response in cases of typical Menière's disease is presumed to be caused by a temporary reduction of endolymphatic hydrops by acute systemic diuresis The fact that the furosemid test results were positive indicates the feasibility of utilizing the test for diagnosing endolymphatic hydrops

Table I *The effect of furosemid on caloric response of diseased ear in the five groups of labyrinthine disease*

Furosemid effect	Positive cases	Negative cases	Total
Typical Meniere s disease	74 (80 %)	19	93
Atypical Meniere s disease	2 (6 %)	32	34
Labyrinthine syphilis	5 (42 %)	7	12
Sudden deafness	3 (27 %)	8	11
Labyrinthitis	0	11	11
			161

Typical Meniere's disease

The furosemid test yielded positive results in 80 % while in 20 % the results were negative and the clinical features characteristic These entities may be regarded as pseudo-negative cases, but treated as positive It has been pointed out by several authors that Meniere's disease might affect the bilateral labyrinth in 9 to 14 % (Karmody & Schuknecht, 1966, Enander & Stahle, 1967) In this study, 8 cases (9 %) showed positive findings in both ears The incidence of bilateral involvement can, however, be presumed in more than 9 %, as there are cases progressing into bilateral lesion, and thus a pseudo-negative value would be demonstrated in the unaffected ear

Fluctuating hearing loss has been regarded by many investigators as a characteristic clinical feature of Meniere's disease For example, the glycerol test (Klockhoff & Lindblom, 1966) has been reported as being effective only for patients with fluctuating hearing loss and not for those with ' flat loss" of the "burned out" cases Meniere's disease is not restricted to those with a fluctuating hearing loss only In the present series, there were a few patients with the "burned out ' type who responded positively in hearing tests Concerning caloric response, almost all of these ' burned out" cases yielded positive values The transient fluctuation caused by a single dose of furosemid or glycerol does not indicate a limit threshold of hearing improvement brought about by treatment As

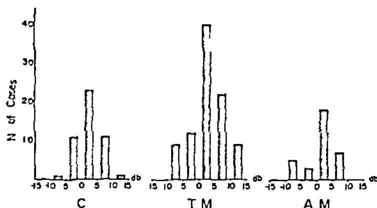


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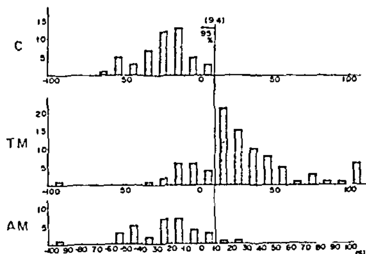


Fig 2 Changes in caloric response following furosemid application Percentage of increase or decrease in maximum velocity is demonstrated in each group Abbreviations as in Fig. 1

lymphatic hydrops This is supported by the histological findings that in Meniere's disease it is the cochlear duct which is chiefly distended (Hallpike & Cairns, 1938)

Labyrinthine syphilis

In 47 % of patients with syphilis, positive effects were observed with regard to detection of endolymphatic hydrops Further, 3 out of the 5 positive cases were bilateral occurrences Karmody & Schuknecht in 1966 demonstrated pathology to be caused by primary osteitis of the otic capsule with secondary hydrops in patients with syphilis Also, Pulec & House in 1973 reported that 7 % of all patients with Meniere's disease had a history of syphilis As the results in this paper show, it is assumed that a higher incidence of syphilitic hydrops can be demonstrated using the furosemid test as patients with syphilitic hydrops responded well to the diuretic effect of furosemid

Sudden deafness

In 3 out of 11 patients with a sudden onset of unilateral acute hearing loss accompanied by a single attack of vertigo, i e sudden deafness, detection of hydrops was demonstrated This would appear to support the theory that hydrops can be included in some cases of sudden deafness If the furosemid test gave positive results, then the decompressive treatment would be taken into consideration even in the cases of sudden deafness

Labyrinthitis

Results were all negative Radical surgery or tympanoplasty (type III or IV) was performed on 9 patients in the labyrinthitis group Karmody & Schuknecht (1966) stated that inflammatory disorders do cause endolymphatic hydrops in serous and suppurative labyrinthitis With regard to the latter, a discrepancy exists between these authors' findings and our results

CONCLUSION

In clinical practice, the furosemid test appears to be a good prognostic method for the medical and surgical treatment of not only typical Meniere's disease but also the other labyrinthine disorders

ZUSAMMENFASSUNG

Bei intravenoser Einspritzung von Furosemid, einer diuretischen Droge, stieg die kalorische Reaktion durch die Dehydration, als der Hydrops im inneren Ohr bei Meniereschen Krankheit beseitigt wurde Dieser Furosemid test wurde an 161 Patienten mit Schwindel [illegible]

[illegible] Syphilis und bei 3 von 11 Patienten (27 %) mit plotzlicher Taubheit, wurde die positive beobachtet Kein positiver Fall mit Labyrinthitis konnte nachgewiesen werden.

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STIMULATION OF THE VESTIBULAR RECEPTOR BY MEANS OF STEP TEMPERATURE CHANGES DURING CONTINUOUS AURAL IRRIGATION

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Abstract A technique for rapid, balanced hot/cold stimulation of the vestibular receptor is presented During continuous aural irrigation the temperature of the irrigation fluid is switched between hot and cold values at times computed according to a mathematical model of heat conduction in the labyrinth area As a result, the induced temperature difference across the lateral semicircular canal describes an approximately sinusoidal time course, reaching peak values of equal magnitude but opposite sign Application of the test to 32 clinical subjects demonstrated that the heat conduction model and the analysis used in timing the sequence of thermal pulses was accurate We expect that, with further refinements, the new technique will prove superior to conventional caloric test methods in the detection and measurement of subtle as well as gross abnormalities of the vestibular system

The technique recommended by Fitzgerald & Hallpike (1942) for performing the caloric test is currently in widespread use as the principal clinical method for testing vestibular function Four separate irrigations are applied (cold left, cold right, hot left, hot right) using a high flow rate and carefully controlled irrigation temperatures with the intention of producing independent stimulations that are all of equal strength Fitzgerald and Hallpike noted the time taken for a single test was about 4 min and advised an interval between tests of at least 5 min, thus requiring a minimum of 31 min to complete the series of tests We feel the prolonged individual stimulations produced by this technique are inappropriate for routine clinical testing and the requirement of long waiting periods between irrigations causes the test procedure to be cumbersome and inefficient

When applying the caloric test it is important to be aware that profound modifications of the nystagmus response may occur in the presence of sustained uniform stimulation (Proctor et al, 1972) An example of such response modification is the fact that caloric-induced nystagmus normally ends long before the inciting physical stimulus has subsided Although Fitzgerald & Hallpike (1942) found that among normal subjects with eyes open nystagmus ended after 1 2 to 2 4 min, temporal bone temperature measurements in living human subjects have shown that significant thermal disturbance continues far beyond the time nystagmus ceases (Kleinfeldt & Dahl, 1969, 1970) and may in fact persist beyond 10 min (Cawthorne & Cobb, 1954) In a recent nystagmographic study Hood (1973) found evidence indicating that a thermal stimulus capable of producing nystagmus will persist for at least 11 min and remarked that such a residual stimulus might react with responses to successive caloric irrigations

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of the thermal stimulus may be explained by an adaptive process of the vestibular neural elements, brought into play during sustained cupular deviations (Aschan & Bergstedt, 1955; Young & Oman, 1970, Malcolm, 1970, Hood, 1973) During normal head movements semicircular canal cupulae are deviated only transiently and then quickly returned to their resting position In contrast to this normal situation the prolonged and unphysiologic cupular deviation caused by the conventional caloric test produces a nystagmus which is progressively diminished through adaptation and then terminated altogether, even while the thermal stimulus maintains cupular displacement Aschan & Bergstedt (1955) showed that secondary nystagmus, which may be taken as a manifestation of the adaptive process, depended not upon cupular deviation per se but rather upon the length of time such deviation was maintained Thus the duration of the nystagmus reaction may be viewed as being determined by two opposing factors, the sensitivity of the vestibulo-ocular reflex and the degree of adaptation which takes place

The above considerations, coupled with the demonstration by Henriksson (1956) and others t nystagmus intensity measurements are ch better correlated with vestibular stimulus intensity than is the duration of the reaction have led most clinicians to base their interpretation of the caloric test upon that period when maximum nystagmus intensity is expected Thus there does not appear to be any useful purpose in maintaining thermal stimulation beyond the time when maximum response intensity has been established Furthermore, the prolonged durations of conventional stimulations are usually a source of discomfort and fatigue to patients as well as inconvenience to those performing the test

Since small changes in magnitude of the caloric stimulus can be expected to produce significant alterations in nystagmus intensity (Henriksson, 1956) it would be important to consider whether unintended variations in magnitude might be expected to occur among the four stimuli produced by the conventional Fitzgerald-Hallpike irrigation method Although a number of investigators have studied the caloric stimulus by means of temporal bone temperature measurement, only in the investigation of Dohlman (1925) was the question of variability of stimulus magnitude examined He reported variations in heat transmission from directing the irrigating stream at different parts of the aural canal Other elements of the irrigation technique which might contribute to variability of stimulus magnitudes are entrapment of air in the aural canal during irrigation, changes in head position relative to the gravity vector (Brünings, 1911, Proctor, 1974) and the quantity of water remaining in the canal following irrigations Therefore unless great care is exercised, reliability of the conventional caloric test may be impaired by inadvertent variations among stimulus magnitudes

An interesting alternative caloric stimulation method was reported by Gates et al (1970), who employed an air caloric stimulator to produce a continuously varying temperature at the aural canal Young (1972) used this method to study the relationship between thermal stimuli and vestibular responses

The present report describes a new caloric test method in which the temperature of a continuous aural irrigation is abruptly switched between hot and cold values at times computed according to a mathematical model of heat conduction in the labyrinth area The new method provides selected peak stimulus values of equal magnitude but opposite sign, with rapid removal of the thermal stimulus at the conclusion of each test

THEORETICAL CONSIDERATIONS

Aural irrigation with cold or hot water produces a temperature gradient across the labyrinth attended by changes in endolymph density, making possible the generation of forces acting to displace the endolymph-cupula system (Fig. 1) and thereby stimulate the vestibular receptor The lateral semicircular canal is primarily re-

sponsible for the nystagmus response and the temperature gradient across this canal may be taken as an approximation of the physical stimulus acting upon the receptor, so long as the orientation of the semicircular canal with respect to gravity is controlled (Young, 1972) In our calculations we have ignored the fact that only the component of the temperature vector orthogonal to the gravity vector contributes to endolymph displacing forces, and we have assumed the labyrinth area can be treated as a solid of uniform thermal characteristics

The time course of the temperature difference across the lateral semicircular canal (ΔT) resulting from a varied irrigation temperature may be calculated from a model assuming unidirectional conduction of heat through a homogeneous solid (Schmaltz, 1932, Young, 1972) With this model standard formulas (Carslaw & Jaeger, 1959) show that beginning irrigation with water at aural canal temperature plus T_1 degrees produces a temperature gradient after t sec of

$$dT/dx = \frac{T_1}{\sqrt{\pi a t}} e^{-x^2/4at} \tag{1}$$

The temperature difference across the canal (ΔT), is simply the gradient dT/dx times the distance across the canal, L Therefore,

$$\Delta T = \frac{L T_1}{\sqrt{\pi a t}} e^{-x^2/4at} \tag{2}$$

Where

T_1 = difference between irrigation water temperature and initial aural canal temperature in degrees, centigrade In our method T_1 has a value of 44°–37° = 7° (centigrade)

L – distance across lateral semicircular canal, millimeters

ΔT – temperature difference across lateral semicircular canal, degrees centigrade

a = thermal diffusivity of temporal bone surrounding lateral semicircular canal, mm²/sec

x – distance between external aural canal and center of lateral semicircular canal, millimeters

t = time elapsing from onset of irrigation, seconds

e natural logarithm base

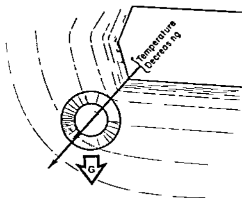


Fig 1 The temperature gradient field caused by hot irrigation of the right aural canal is represented by fine interrupted lines An arrow indicates decreasing temperature across the lateral semicircular canal With the subject's head in standard testing position gravity is directed as shown by the large open arrow A difference in endolymph density indicated by shading would act to move the endolymph in a counterclockwise direction thereby producing nystagmus directed toward the stimulated ear Cold irrigation would cause the opposite effect.

In the present study we used values for the above parameters determined by Young (1972) $L = 6$ mm, $a = 0.25$ mm²/sec, and $x = 10.5$ mm With a, L, x and T_1 constant, eq (2) may be simplified to read

$$\Delta T = \frac{-A}{\sqrt{t}} e^{-B/t} \tag{3}$$

where A and B are constants This equation predicts that for a continued irrigation the absolute magnitude of the temperature difference (ΔT) increases to a maximum value and then diminishes For example, continuous irrigation with water at 44°C would result in an absolute maximum ΔT value of 1.93 C after 220 sec

However, we have chosen to interrupt the 44°C irrigation by quickly changing the temperature of the irrigation to 30°C and eq (3) must be expanded to account for this condition

of the thermal stimulus may be explained by an adaptive process of the vestibular neural elements, brought into play during sustained cupular deviations (Aschan & Bergstedt, 1955, Young & Oman, 1970, Malcolm, 1970, Hood, 1973) During normal head movements semicircular canal cupulae are deviated only transiently and then quickly returned to their resting position In contrast to this normal situation the prolonged and unphysiologic cupular deviation caused by the conventional caloric test produces a nystagmus which is progressively diminished through adaptation and then terminated altogether, even while the thermal stimulus maintains cupular displacement Aschan & Bergstedt (1955) showed that secondary nystagmus, which may be taken as a manifestation of the adaptive process, depended not upon cupular deviation per se but rather upon the length of time such deviation was maintained Thus the duration of the nystagmus reaction may be viewed as being determined by two opposing factors, the sensitivity of the vestibulo-ocular reflex and the degree of adaptation which takes place

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from the above irrigation sequence form a smooth curve as shown in Fig 2 In agreement with temporal bone temperature recordings (Dohlman, 1925, Schmaltz, 1932, Cawthorne & Cobb, 1954, Kleinfeldt & Dahl, 1969, 1970, Young, 1972) our calculations indicate a latency between thermal events in the aural canal and their resultant effects acting upon the lateral semicircular canal Thus a significant temperature difference does not appear until about 15 sec after the beginning of the first irrigation Also, despite switching from hot to cold water at 38 sec the effect of the hot irrigation continues to predominate until a peak value is reached ($-\Delta T^*$) at 58 sec Similarly the cold irrigation ends at 96 sec ($t_1+t_2=96$ sec) but $+\Delta T^*$ does not occur until 117 sec

After the final hot irrigation is terminated temperature at the aural canal surface returns slowly toward normal body temperature (Henriksson, 1956) Since the specific time course of this temperature change is unknown there is uncertainty concerning the final course of ΔT An approximation was achieved by calculating the final course of ΔT according to a continuation of the irrigation with temperature at 37°, following final (hot) irrigation times of 12 and 20 sec The true course of ΔT should at first lie between these two cases (interrupted lines in Fig 2) However after about 150 sec our estimations may be inaccurate

In the above discussion we explained the derivation of a new caloric test method that produces rapid, balanced hot/cold stimulation of the vestibular receptor Next we would like to report the results of a preliminary study in which we tested the accuracy of our calculations and evaluated the practicality of the new stimulation method The irrigation sequence of the new test (44° × 38 sec, 30° × 58 sec, 44° × 12 sec) was applied to 32 unselected patients referred to the clinical vestibular laboratory We expected the intensity and direction of nystagmus resulting from this irrigation procedure would follow a pattern corresponding to the ΔT curve of Fig 2 Water was directed against the postero-superior part of the aurral canal under visual control from an irrigation handle (Proctor & Byrnes, 1973) containing a temperature sensor Continuous recordings from the sensor enabled us to monitor irrigation temperatures and switching times A valve connected two water baths with external circulation to the irrigation handle, providing a means for quickly changing the temperature of the irrigation water from 44° to 30° and vice versa Although this apparatus requires further development we were able to control irrigation temperature with accuracy sufficient for the present study

Responses were recorded electronystagmographically (ENG) using a Beckman type R dynograph and commercial silver silver chloride skin electrodes at the outer canthi We used 10 mm/sec paper speed, 3 sec time constant and "slow" setting for filters Calibration (10° eye movement = 10 mm pen displacement) was adjusted immediately before the series of caloric stimulations, which were performed with the patient recumbent and head raised 30° above horizontal Subjects were instructed to keep their eyes closed, and were "alerted" with arithmetic tasks Testing was carried out in a dimly lit room and caloric stimulations were preceded by oculomotor and positional tests To compare the new sinusoidal bithermal caloric (SBC) test with conventional stimulations we administered separate hot stimulations (44° × 30 sec) to all subjects

ENG recordings were analysed by determining mean slow phase eye speed for each 5 sec of the response and plotting values as shown in Figs 4 5 7 A nystagmus beat was scored whenever there was interruption of eye movement in one direction by movement of greater velocity in the opposite direction (Proctor et al, 1972) Beats were assigned positive and negative values when the fast phase of the beat was directed toward the stimulated or opposite ear, respectively If only a single beat occurred in one direction during 5 sec it was not plotted

Average slow phase eye speed was calculated for the 20 sec period during which response to the hot mode stimulus of the SBC test was

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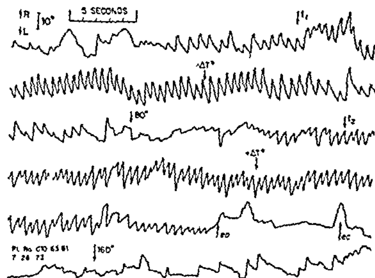


Fig 3 Patient A Eye movement tracing from subject with normal responses, showing buildup and decay of nystagmus to same and opposite sides following sinusoidal bithermal caloric (SBC) stimulation Irrigation was begun at 44° and switched to 30 after 38 sec (t_1) 58 sec later (t_2) irrigation temperature was returned to 44 for 12 sec. Note peak nystagmus intensities at 58 sec ($-\Delta T^*$) and 117 sec ($+\Delta T^*$), null period at 80 sec, and third phase nystagmus after 160 sec *eo*, *ec*, eyes open, closed. Hatch marks indicate onset of fast phase of nystagmus beats

strongest (usually 45 to 65 sec) and for the time period when response to the cold mode stimulus was strongest (usually 105 to 125 sec) Differences in excitability between labyrinths and between right-beating versus left-beating nystagmus were determined in relation to total excitability as recommended by Jongkees & Philipszoon (1964)

Unilateral hypoexcitability score

$$\frac{(1+2)-(3+4)}{(1+2+3+4)} \times 100\%$$

Directional preponderance score

$$\frac{(2+3)-(1+4)}{(1+2+3+4)} \times 100\%$$

Where

1 = Hot mode stimulus, left ear
2 = Cold mode stimulus, left ear
3 = Hot mode stimulus, right ear
4 = Cold mode stimulus right ear

Since we have not as yet determined normative performance for the SBC test, we arbitrarily applied the same normative values to our data as those reported by Jongkees & Phillipszoon (1964) for the conventional caloric test These authors used a 30 sec irrigation and recorded responses with the subjects eyes closed From calculations of maximum slow phase eye speed they determined the limits of normal for unilateral hypoexcitability (UH) scores were ±15% and for directional preponderance (DP), ±18%

RESULTS

According to the values cited above, 13 patients had normal SBC responses, 6 had UH alone, 3 had DP alone, and 10 showed combined abnormalities

A response typical of the 13 normal subjects, though exhibiting *more intense nystagmus* than some, was recorded from a 79 year-old female who complained of intermittent mild vertigo, but whose oculomotor and vestibular tests revealed no abnormalities (Patient A, Fig 3) As can be seen, nystagmus began toward the irrigated ear, increased progressively in intensity to reach a maximum at approximately the time of maximum stimulation predicted from our calculations ($-\Delta T^*$ at 58 sec) and then progressively diminished After a brief null period nystagmus appeared in the opposite direction, again reaching maximum intensity at approximately the predicted time ($+\Delta T^*$ at 117 sec) A plot of average slow phase eye speed for each 5 sec interval during this response is shown in Fig 4 There is an impressive correspondence between the be-

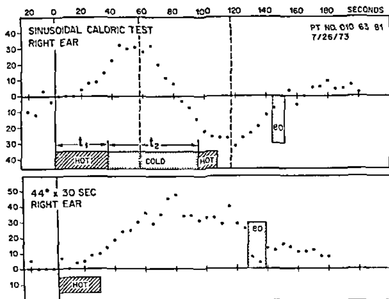


Fig 4 Patient A *Upper figure* Plot of mean slow phase eye speed during nystagmus response shown in Fig 3 *Ordinate* Slow phase eye speed, degrees per second Positive and negative values indicate nystagmus directed toward the stimulated and opposite ear, respectively Shaded areas labeled 'hot' and 'cold' indicate irrigation sequence Dashed vertical lines show times of predicted peak temperature differences (58, 117 sec) *eo*, eyes open *Lower figure* Plot of mean slow phase eye speed during nystagmus response to conventional hot irrigation of same ear Time is scored on abscissa with same scale as upper figure

havior of the temperature difference curve (ΔT, Fig 2) and the nystagmus intensity plot (Fig 4) When nystagmus was well developed, even in subjects having abnormal responses, there was *usually a close correspondence between the time* of occurrence of peak slow phase eye speed and predicted peak temperature difference values, $\pm\Delta T^*$ Table I summarizes the findings among the plots of 75 hot and cold mode responses where intensity rose to a discernible peak value Note a tendency for late-occurring peak intensities to be correlated with normal responses and early peak intensities with abnormal responses

We usually examine each subject's temporarily opened eyes at some time during routine caloric testing to confirm the direction of nystagmus and to test 'fixation suppression" (Ledoux & Demanez, 1970) Examples of *temporary diminution in nystagmus intensity* resulting from this maneuver can be seen in Fig 4 (eo, lower figure) and Fig 7 (eo, middle figure) However, subjects were instructed to keep their eyes closed during the SBC test, the "*eyes open*" *period in the SBC response of* patient A (Fig 3 Fig 4, upper figure) was an inadvertent lapse in our routine

As explained above, we considered the response of patient A to the new test was in agreement with our predictions as regards direction and peak intensity of nystagmus However, we were surprised to observe that following *subsidence of the second (cold mode) nystagmus* phase there appeared a third nystagmus phase, beating once more toward the irrigated ear (Fig 3, Fig 4, upper figure) A similar third phase nystagmus appeared in 20 of the 32 *subjects in this study In 5 cases* the phenomenon could be attributed to the return of spontaneous nystagmus which had been beating toward the irrigated ear before stimulation was applied (Fig 6C, Fig 7, top figure) In the *remaining 15 cases no clinical explanation* was

Table I *Time of occurrence of peak slow phase eye speed relative to predicted peak stimulus intensities*

Negative time values indicate peak nystagmus intensity occurred before peak stimulus intensity (Not all responses showed a discernible peak intensity)

	< ±5 sec	−5 to −15 sec	+5 to +15 sec
Normal responses (13 subjects)	39	1	8
Abnormal responses (19 subjects)	36	10	3

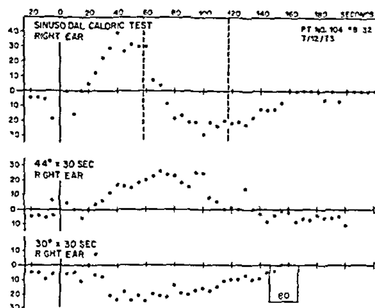


Fig 5 Patient B Mean slow phase eye speed plots of nystagmus produced by sinusoidal and conventional stimulations of the right ear The new SBC test (upper figure) and the conventional test (middle and lower figures) both indicate normal responses from the right vestibular receptor *eo*, eyes open Conventional cold stimulations were performed on only a few subjects in this study Note that a spontaneous nystagmus directed away from the stimulated ear was present before each irrigation began

evident Among most of our subjects nystagmus either ended or reversed direction before about 160 sec

The frequent occurrence of third phase nystagmus could mean the final hot irrigation is too long or improperly terminated, causing a temperature difference to appear and the vestibular receptor On the other the physical stimulus may be essentially *at about 180 sec and nystagmus appearing* at this time may be related to the vestibular adaptive process discussed above (Aschan & Bergstedt, 1955, Young & Oman, 1970, Malcolm, 1970, Hood, 1973) Rapid termination of the thermal stimulus offers certain practical advantages and the possibility of new approaches to studying vestibular responsivity However, precise control of stimulus termination must await further studies concerning the time course of temperature within aural canal and labyrinth following the irrigation sequence

When the expected nystagmus is feeble, *poorly formed, or obscured by the presence of* spontaneous nystagmus there is often uncertainty whether a given sequence of eye movements can be taken as proof of a response to vestibular stimulation (Proctor, et al, 1972)

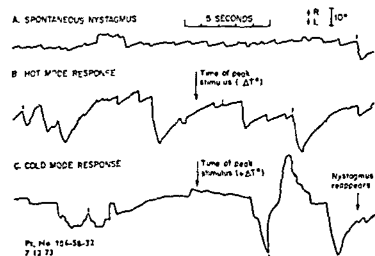


Fig 6 Patient B Eye movement tracings from subject who showed spontaneous nystagmus to the left with eyes closed before testing (A) and weak responses to thermal stimulation of the left ear Nystagmus intensity increased during the hot mode stimulus (B) and diminished during the cold mode stimulus (C) The slight increase in nystagmus intensity during the hot mode stimulus and temporary cessation of spontaneous nystagmus during the cold mode stimulus are both taken to indicate the vestibular receptor was responsive Note the return of nystagmus as the cold mode stimulus diminishes (C)

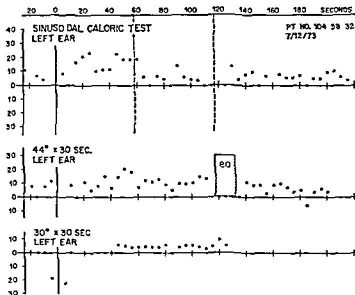


Fig 7 Patient B Mean slow phase eye speed plots reveal diminished nystagmus responses to sinusoidal and conventional stimulations of left ear Note (top figure) increased nystagmus intensity during hot mode stimulation and temporary cessation of nystagmus at the time of peak cold mode stimulus intensity (dashed vertical line at 117 sec)

This difficulty is of great practical importance since often the principal question posed by the clinician is simply whether or not the vestibular receptor responds to stimulation Thus it is significant that only 4 instances occurred wherein a nystagmic response was well formed and easily identified following conventional caloric stimulation but not recognizable with confidence following corresponding SBC stimulation in the same subject However, among most subjects in this admittedly small sample it appeared the SBC stimulus acted long enough that even feeble responses were clearly recognized

To illustrate this point, the responses are shown of a 34-year-old female patient suffering from vertigo, tinnitus, and profound hearing loss in her left ear, of unknown etiology (patient B, Figs 5–7) She had spontaneous nystagmus toward the left with eyes closed and direction-changing positional nystagmus A strong nystagmus developed toward the right and then toward the left following sinusoidal stimulation of the right ear, symmetrical responses were also produced by standard hot and cold irrigations of the right ear (Fig 5) We concluded the responses of the right vestibular receptor were within normal limits

When the left ear was tested there was a weak but clearly observable response during the hot mode of the sinusoidal caloric test and a brief cessation of (spontaneous) nystagmus during the cold mode (Figs 6 and 7) There was a somewhat less distinct response to conventional hot irrigation of the left ear, following conventional cold irrigation the question of a response was even less clear since spontaneous nystagmus was not stopped during the time of maximum stimulus strength We conclude in this instance, where responses were weak and spontaneous nystagmus was present, the question of whether the left vestibular receptor was responsive or in fact unresponsive was possibly settled more clearly by the SBC test than by the conventional test

Responses to the new SBC test were usually less intense than were those to conventional stimulation (Table II) Average slow phase eye speed during maximal 20 sec periods of all responses to SBC hot mode stimuli was 16 95°/sec, compared with an average value for all responses to conventional hot stimuli of 22 41°/sec The difference in mean response intensities is partly explained by the fact that the SBC stimulus declines more rapidly after reaching a peak value than does the conventional stimulus Patients reported less intense vertigo and definitely less discomfort SBC

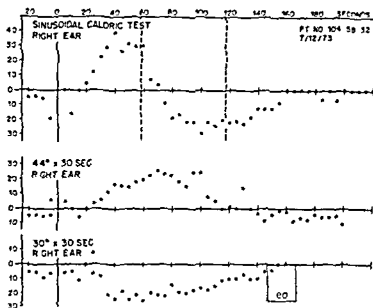


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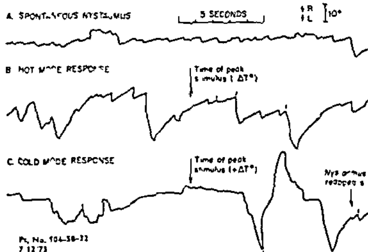


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der Spülungsflussigkeit zwischen heiss und kalt gewechselt Die Wechselzeiten der Temperaturen werden nach einem mathematischen Modell der Warmeleitung in der Labyrinthgegend geregelt Daraus ergibt sich ein annahernd sinusformiger Zeitverlauf des herbeigefuhrten Temperaturunterschieds quer durch das Labyrinth Diese neue Technik ist eine Verbesserung der konventionellen Methoden denn die Reize sind kurzer und deshalb physiologischer, die Prufung wird schneller durchgefuhrt, und bei gewissen Reizstarken wird die Moglichkeit unabsichtlicher Variationen vermindert 32 klinische Testpersonen wurden untersucht Diese Untersuchung bestatigte die Genauigkeit des Wärmeleitungsmodells und die Analyse, die angewendet wurde, um die Folge der thermischen Anregungen zeitlich zu regeln. Die Untersuchung wies auch darauf hin dass feinere sowie allgemeinere Eigenschaften der vestibularen Reizbeantwortung mit der neuen Prufung deutlicher und zuverlassiger festgestellt werden durften als mit konventionellen Methoden

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MUCOCILIARY WAVE PATTERN

A Comparative Analysis of Extracellular and Intracellular Activities

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Abstract Experimental in vitro studies have been made on the intracellular electrical activity and the extracellular wave movements of ciliary cells in rabbit trachea The following results were obtained

1 Surface light reflections from the mucous layer and from the ends of moving cilia showed about the same frequency and amplitude pattern as the intracellular action potential oscillations at 40 C and 50 C. At lower temperatures however there was a considerable discrepancy between them

2 Surface light reflections from mucociliary wave movements had the same frequency in two areas 20 μm apart but amplitude variations were out of phase

3 Intracellular oscillations with a frequency of 18–21 sec and a maximum amplitude of 1 mV have been Amplitude variations indicating rhythmical frequency variations have also been recorded

4 The mean frequency of the intracellular action potential oscillations showed no remarkable differences at 20 C 30 C 40 C and 50 C

The mucociliary activity of respiratory mucous membranes has attracted increasing interest during the past century In spite of all efforts many fundamental problems regarding the mechanisms of movement, the energy supply, and the coordination, interaction and control of ciliary activity remain unsolved The capacity of mechanical transportation of secretions and particles has been observed both *in vitro* and *in vivo* with the use of various methods The local activity brought about by the lashing movements of cilia has been thoroughly investigated with a variety of indirect methods based on surface light reflections The intracellular electrical activity of single cell organisms and metazoans with motile cilia has hitherto been the object of only a few investigations (Horridge, 1965) Håkansson & Toremalm (1966*a*) described the profile of intracellular potentials at different levels of the rabbit tracheal wall in *in vitro* experiments from the secretion layer down to the smooth muscle layer beneath the basal membrane It was also possible to record potential oscillations, interpreted as superimposed action potentials, using a capillary microelectrode placed inside ciliary cells (Håkansson & Toremalm, 1966*b*) These oscillations had a frequency pattern which was very similar to that obtained indirectly via surface light reflections. Amplitude variations were recorded intracellularly with patterns very like those seen in surface reflection studies The frequency and amplitude variations obtained via surface reflections are partly due to a double origin of the reflections, i e from the mucous waves and from the ends *of moving cilia (Toremalm et al, 1974) The* aims of the present experimental *in vitro* investigation on rabbit trachea have been (1) *to* analyse the amplitude and frequency variations of recordings obtained by the surface light reflection method, (2) to analyse and compare *simultaneous recordings of surface light reflec*tions from two separate areas close to each other in the same tracheal specimen, and (3) to com-

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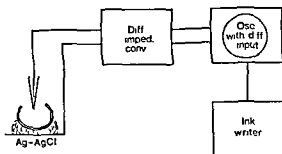


Fig 1 Block diagram showing the experimental arrangement for the recording of intracellular action potentials in ciliary cells of rabbit trachea (for details see text)

pare at different temperature levels frequency and amplitude patterns of potentials recorded from intracellular electrodes and of surface light reflections

METHODS

Recording of surface light reflections

The method used for recording light beam reflections from respiratory epithelium has been described elsewhere (Mercke et al, 1974) A photomultiplier with a diaphragm aperture of 0 1 mm was used for transducing surface light reflections detected in one ocular of a binocular microscope For some of the experiments two photomultiplier units were used, one attached to each microscope ocular

A computer has been used for Fourier analysis of some of the recorded wave forms (Fig 3)

For analysis of interference phenomena two frequencies have been mixed by the computer, one basic frequency of 12 Hz and one other frequency of 11, 10, 9, 8 or 7 Hz (Fig 5)

Recording of intracellular electrical activities

A block diagram of the experiment is shown in Fig 1 Glass microelectrodes filled with 3 M KCl solution and with an impedance of 10 MΩ and an external tip diameter of less than 0 5 μm were used The electrodes were connected to a micromanipulator allowing penetration through the mucus and the membrane of the ciliated cell This method and procedure has also been described elsewhere (Håkansson & Toremalm, 1966*a* and *b*) The recording of electrical potentials was made between the outside of the tracheal surface (where the Ag–AgCl-electrode made contact with the surface via cotton wool soaked in Ringer solution) and the glass electrode inside the trachea Care was taken to insulate the chloride surface from the surface of the metal in both electrodes Since the experiments were performed at above 90 % relative humidity potential changes due to evaporation were not seen A maximum of 5 mV was allowed as microelectrode tip potential The impulses were fed into a transistorized impedance converter with a resistance above 10^3 MΩ and thence into a differential amplifier (Tektronix 3A3) The noise level was 250 μV The potentials were monitored on an oscilloscope (Tektronix 565), permitting accurate management of the micromanipulator With open input and trigger switched to d c, the beam on the oscilloscope moved irregularly but as soon as the tip of the electrode touched the mucus, the beam immediately stabilized With a c coupling, the beam remains steady on the monitor and a jump indicated that the electrode had touched the surface of the mucus The electrode was manually pushed downwards through the mucus, with the trigger in the d c position, a sudden potential drop of about 15–40 mV indicating that a ciliated cell membrane had been penetrated The trigger was then switched to the a c position and the intracellular potentials recorded by an ink-writer (Elema Mingograph 34) after filtering (Krone Hite type 3550)

RESULTS

The frequency and amplitude variations of mucociliary light reflections were recorded with the standardized method previously described in detail (Mercke et al, 1974) An ordinary recording at a temperature of 33 2°C and an environmental air humidity above 90 % is shown at Fig 2 There is a mean frequency of 12 waves/sec and fairly regular amplitude variations with a rhythm of about 1 5 cycles per sec

The recording at Fig 2 has been analysed by computer in order to find the main wave fre-



Fig 2 Recording of mucociliary light reflections from the rabbit trachea at a temperature of 33 2°C and a relative humidity above 90 %

quency and the scatter (Fig 3) In this case it is found to be 12±2 waves/sec Frequencies within the range of 7–10 waves/sec are also seen The peak to the extreme left in Fig 3 probably represents the rhythmic activity of the smooth muscles of the tracheal wall, approximately 0 5 contractions per second

A more complex pattern of frequency and amplitude variations is usually seen at temperatures some degrees below body temperature The two upper recordings at Fig 4 were obtained at a temperature of 23 8°C The frequency is calculated to be 428 waves/min However, it is not so easy to calculate the frequency from these recordings as compared with the recording in Fig 2 The amplitude variations are also very irregular The lower recording at Fig 4 obtained at 30 1°C shows a less complicated pattern with an average frequency of 11 waves sec (618 waves/min) The amplitude variations are less irregular and the recording is clearly composed of more than two separate rhythms

In order to produce a prototype of mixed wave patterns on a mathematical basis we have fed a computer with two frequencies The results appear in Fig 5 where frequencies in the range 7–11 Hz have been separately added to a basic frequency of 12 Hz The resulting patterns should be compared with the light reflection recordings at Figs 2 and 4

Simultaneous recordings of mucociliary wave reflections from two tracheal areas from the same specimen and only 20 μm apart are seen at Fig 6 (recording A and B respectively) They were obtained at a temperature of 40 C and a relative humidity above 90 % This recording was made by putting a photomultiplier unit on each ocular of the microscope There is no obvious frequency difference but the pattern of the amplitude variations differs slightly

A typical recording of surface light reflections at a temperature of 33 4°C and a relative humidity above 90 % is seen at Fig 7 (above) The

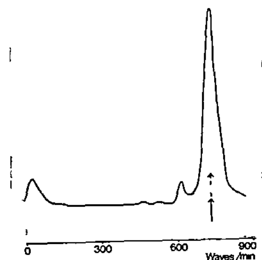


Fig 3 Computer analysis of the mucociliary light reflections shown at Fig 2 The main frequency is 12 waves/sec (continuous arrow) and the scatter is ±2 waves/sec The calculated average frequency is 12 waves/sec (broken arrow)

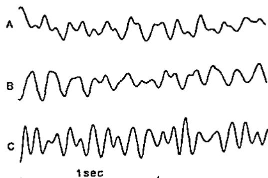


Fig 4 Recording of mucociliary light reflections from the rabbit trachea at 23 8°C (*A* = 428 waves/min), 23 8°C (*B* = 428 waves/min) and 30 1°C (*C* = 618 waves min) Relative humidity above 90 %

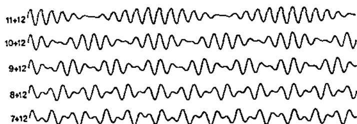


Fig 5 The result of mixing a basic frequency of 12 Hz with various lower frequencies The wave forms were generated by computer

frequency is 13 waves/sec and the amplitude variations are regular A typical recording of intracellular potential oscillations from a capillary microelectrode placed in the ciliary cell layer is seen at Fig 7 (below) Here the temperature is 38°C and the relative humidity above 90% The frequency is 20–21 oscillations/sec The maximum amplitude reaches about 1 mV The amplitude variations are less regular

Fig 8 illustrates a comparison of the frequencies and amplitudes of oscillating intracellular potentials (*I*) and surface light reflections (*R*) The recordings are obtained from different tracheal specimens at 20°C, 30°C, 40°C and 50°C and with a relative humidity in the surrounding air above 90% It is noted that the intracellular potential oscillations have a frequency of 18–20 oscillations/sec at all four temperatures The surface light reflections had a frequency of 18–19 waves/sec at 40°C and 50°C, which decreased to about 12 waves/sec at 30°C and to about 6 waves/sec at 20°C Amplitude variations are seen in the intracellular potential recordings as well as in surface light reflections The variations of the intracellular recordings are fairly regular at all four temperatures

DISCUSSION

Most methods used for the study of mucociliary wave patterns in the respiratory tract can be classified as indirect, i e a recording of secondary movements in the mucous layer Such methods are not by themselves enough for the investigation of the many still remaining problems regarding mechanical, electrical and biochemical processes in ciliary cells It is therefore valuable to develop and try other experimental approaches

It has been possible to obtain intracellular action potentials from among others ciliated metazoan organisms (Horridge, 1965) and also to record an oscillating electrical activity with a rather high frequency in more complex systems of coordinated ciliary activity (Håkansson & Toremalm, 1966*a* and *b*)

An ordinary recording of mucociliary light reflections obtained by our method is illustrated in Fig 2 A mean frequency of 12 waves/sec can easily be calculated from the recording Typical amplitude variations with a rather regular pattern are seen, indicating the presence of at least two mixed frequencies This fact is

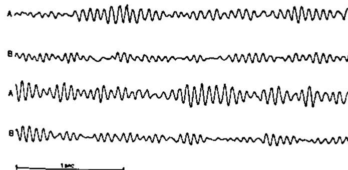


Fig 6 Simultaneous recordings (*A* and *B*) of mucociliary light reflections from two separate areas 20 μm apart Temperature 40°C, relative humidity above 90%



Fig 2 Recording of mucociliary light reflections from the rabbit trachea at a temperature of 33 2°C and a relative humidity above 90 %

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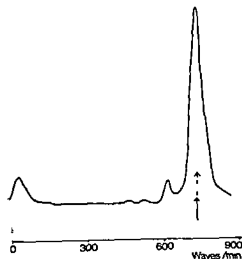


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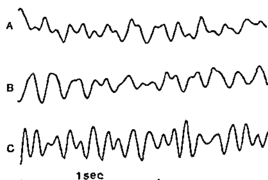


Fig 4 Recording of mucociliary light reflections from the rabbit trachea at 23 8°C (*A* = 428 waves/min), 23 8°C (*B* = 428 waves/min) and 30 1°C (*C* = 618 waves/min) Relative humidity above 90 %

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How are the intracellular action potentials influenced by temperature changes? Some experiments have been done at 20°C, 30°C, 40°C and 50°C as seen in Fig 8 (*I*) For comparison recordings of surface light reflections from other specimens at the same temperatures are shown in Fig 8 (*R*) The frequency of the electrical activity is about the same at all four temperatures, 18–20 oscillations/sec The same frequency range was found for the surface light reflections at 40°C and 50°C At lower temperatures, however, there is a great frequency discrepancy between the intracellular and the extracellular activities This is mostly due to the increasing rheological resistance at low temperatures influencing both origins of surface reflections Amplitude variations are also seen in the intracellular recordings This is a very interesting phenomenon, which is not possible to explain on the basis of the present experiments

Further investigations are also planned in order to obtain surface light reflections and intracellular electrical potentials simultaneously from the same tracheal area for different temperature and humidity conditions *In vivo* experiments would also be valuable but it is questionable whether they can be done without disturbances from respiratory and vascular movements

ZUSAMMENFASSUNG

An Trachealpraparaten von Kaninchen sind Versuche betreffs der intrazellularen elektrischen Aktivität und der extrazellularen Wellenbewegungen der Flimmerzellen durchgefuhrt worden Es ergaben sich folgende Resultate

1 Oberflachenlichtreflexe vom Schleimlager und von den Enden der schlagenden Flimmerhaare verliefen nach einem Frequenz- und Amplitudenschema, das mit den intrazellularen Aktionspotentialen bei 40 C und 50°C sehr gut in Einklang stand Bei tieferen Temperaturen wurde hingegen ein bedeutender Unterschied in dieser Hinsicht festgestellt

2 Oberflachenlichtreflexe von mucoziliaren Wellenbewegungen erfolgten mit gleicher Frequenz in zwei verschiedenen Gebieten, die 20 μm von einander lagen, aber die Amplitudenschwankungen waren nicht phasengleich

3 Intrazellulare Schwingungen mit einer Frequenz von 18–21 pro Sekunde und einer maximalen Amplitude von 1 mV sind registriert worden Ausserdem wurden Amplitudenschwankungen festgestellt, die auf rhythmische Frequenzschwankungen zuruckzufuhren sind

4 Die Durchschnittsfrequenz der intrazellularen Aktionspotentiale wies keine bemerkenswerten Veranderungen bei unterschiedlichen Temperaturen von 20°, 30°, 40° und 50°C auf

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also evident from computer analysis of the recordings The main frequency from the sequence recorded at Fig 2 is 12 waves/sec with a scatter of ±2 waves/sec as is shown by the computer diagram at Fig 3 The light reflections arise from two surfaces, i e the secretion layer and the carpet of moving cilia (Toremalm et al, 1974) At body temperature and a relative humidity above 90% both reflections seem to be coordinated and the recordings regular as in Fig 2 At lower temperatures or decreased relative humidity in the surrounding air, rheological forces restrain the cilia and change the shape of the mucous waves The surface light reflections become irregular and the frequency lowered Under such circumstances the recordings may be difficult to interpret Some examples are given in Fig 4 At a temperature of 23 8°C these difficulties are obvious, but at 30 1°C a more even rhythm can be identified even if it is not absolutely regular In a very regular surface light reflection recording (Fig 7, above), only two frequencies appear to be mixed

In order to get a better understanding of our recordings at different temperatures and humidities we have made a mathematical analogue of two frequencies with the aid of a computer In Fig 5, the basic frequency is 12 Hz mixed with a second frequency of 11, 10, 9, 8 or 7 Hz The 12+11 Hz composition is rather similar to the biological recording at Fig 7, above, and the 12+8 or 12+7 Hz rhythm to the lower recording at Fig 4 The resemblance

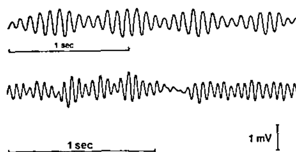


Fig 7 Extracellular light reflection recording at a temperature of 33 4°C (above) and intracellular potential recording from the ciliary cell layer of the rabbit trachea at 38 0°C (below) The frequency is 13 waves/sec and 20–21 oscillations sec respectively In both cases the relative humidity is above 90%

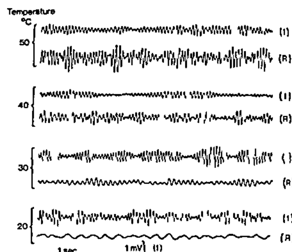


Fig 8 Recordings of intracellular potential variations (*I*) and mucociliary surface light reflection variations (*R*) from different rabbit tracheae at four different temperature levels and a relative humidity above 90%.

is striking, but why is it impossible to obtain a pure sinus recording under optimal conditions? Is there possibly a lack of coordination between adjacent ciliary cells or groups of cells with different frequencies? To answer this, some experiments were carried out with simultaneous recording from two areas in the same tracheal specimen and only 20 μm apart One result appears at Fig 6 The same mean frequency can be calculated from both recordings (A and B) However, the amplitude variations do not coincide in time, indicating a phase shift This may have an extracellular or an intracellular explanation

The next step has therefore been to investigate the intracellular electrical activity This was done using a slight modification of the method described by Håkansson & Toremalm in 1966 A typical pattern of potential oscillations from a ciliary cell is shown at Fig 7, below The frequency here is 20–21 oscillations/sec and furthermore similar amplitude variations are seen as in the case of surface light reflections (cf Fig 7, above) We are not yet able to explain in detail the resemblance between the pattern of surface light reflections and that of the intracellular electrical activity, but it is conceivable that the mechanical activity reflects the electrical intracellular "pacemaker" activity A closer anal

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PHARMACOTHERAPY IN TRACHEAL STENOSIS

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(Received May 29, 1974)

Abstract From our experience in the treatment of immunological diseases with a tendency to the formation of granulomas we conceived the idea of treating pharmacotherapeutically the scar tissue formation in recurrent tracheal stenosis We have combined two medicines with different modes of action, namely, klorokin and penicillamine Two patients with recurrent tracheal stenosis have been treated with remarkable results After several unsuccessful operations it has now been possible to decannulate both of them and enable them to regain a normal way of living

Tracheal stenosis is a disease arising from several causes The treatment is almost invariably surgical, but it is difficult and is frequently distressing for both patient and physician Not infrequently, the patient suffers relapses and has to be reoperated one or more times Despite perhaps many failures, it is nevertheless usually possible to decannulate the patient and to enable him/her to again lead a normal social life The fact that the disease is difficult to treat, has given rise to lively research in this field, and many operational techniques have been developed However, the way in which the stenosis heals does not depend on the operational method alone, but equally on how the wounds heal on the whole The healing of scar tissues in these patients has not infrequently been combined with keloid formation, and it is precisely these patients who suffer from relapses of tracheal stenosis, which make it almost impossible to successfully decannulate them

Experience in the treatment of immunological diseases with a tendency to the formation of granulomas, for example, rheumatoid arthritis and sarcoidosis and pronounced proliferation of connective tissues in systemic sclerosis, has given us the idea of treating pharmacotherapeutically the scar tissue formation in recurrent tracheal stenosis We therefore chose a combination of two medicines with different modes of action, namely, klorokin and *d* penicillamine They have a common characteristic—their effect develops gradually A minimum period of treatment of 3 months is necessary The mode of action of klorokin is manifold It acts as an enzyme inhibitor in several systems Furthermore, the growth of fibroblast cultures is inhibited, as also is collagen synthesis (Haberland et al, 1959) Animal experiments have shown that klorokin inhibits the development of croton oil granulomas (van Cauwenberge et al, 1958) In sarcoidosis a retrogression of cutaneous granulomas has been observed after treatment with klorokin (Shaffer et al, 1953, Klauder, 1953, Soderstrom, 1960, Morse et al, 1961) Klorokin has also a well documented effect on rheumatoid synovitis, causing regression of the inflammation as well as a reduction of the increased sedimentation rate and the rheumatoid factor titers

Penicillamine, on the other hand, also has a multifacetted mode of action It alters the balance between soluble and insoluble collagen in the skin, so that the *soluble fraction* is remarkably increased (Harris & Sjoerdsma 1966) In a carefully controlled study of rheumatoid arthritis, the anti inflammatory effect

of penicillin has been clearly documented It is of special interest that the almost specific granulomas of this disease, the so-called subcutaneous rheumatoid noduli, sometimes disappear during penicillamine treatment, this occurs very rarely, spontaneously (Jaffe, 1968) It has also been found that the concentration of immunoglobulin IgG and IgM in serum decreases during the treatment We have treated 2 patients suffering from frequently recurrent tracheal stenosis, with the above-mentioned medicines, and the results have been so promising that we think they should be reported

Case 1

The patient (born 1905) was injured in a car accident in June 1964 and was tracheotomized and then placed in a respirator for 10 days Nevertheless, she could soon be decannulated and discharged However, 4 months later, breathing difficulties developed and in November 1964 she had to be retracheotomized In December 1964 the tracheal scar tissue was excised, a reconstruction was performed, and a teflon tube was placed in the trachea This tube was retained till September 1965, when it was removed and the patient decannulated However, increased breathing difficulties a week later made it necessary to again insert a cannula Between May 1966 and March 1971 three different attempts were made to decannulate the patient but all without success She then had a silver cannula until January 1973, when she was given klorokin phosphate (Tresochin®) 0 25 g daily and, additionally, d penicillamine (Cuprimine®) 0 25 g daily with successively increasing doses of 0 25 g every week up to 1 5 g daily, which occurred after about 9 weeks Since penicillamine increases the need for pyridoxine, an extra daily dose of 40 mg of this vitamin was given The increased loss of copper, an undesirable side effect of penicillamine forming complexes of chelate with heavy metals, was neutralized by administration of 5 mg $CuSO_4$ daily At the beginning of the treatment an ocular investigation was made and during the treatment blood investigations were performed, including Hb, leucocyte, differential reaction and thrombocytes, alkaline phosphate, transaminases, and protein in urine In this patient no injurious side effects were observed, but minimal proteinuria was noted In May 1973 the patient was decannulated Since then she has had frequent check ups, and has continued to take her medicine No signs of restricturation was observed The tracheostoma has gradually diminished in size and is now almost closed The medication ceased in December 1973 The patient is well and a tracheoscopy has shown a smooth mucous membrane in the trachea without any strictures

Case 2

The patient (born 1912) has had difficulties with chronic stenosing laryngitis since 1952 A pathological investigation was made and an angiofibroma was suspected In June 1954 a laryngofissure with excision of scar tissue was made Postoperatively, probes have been made, but in April 1956 it was impossible to dilate the lumen sufficiently and the patient had to be tracheotomized Between May 1956 and September 1972 laryngofissures had to be made six times because of recurrences and a free skin graft was inserted in the lumen and kept in place by a stent

In January 1973 the stent was removed and the patient was given 0 25 g of klorokin to decrease the granulation tendency Her condition had not improved after 3 months and, consequently, penicillamine was given in doses of 0 25 g daily, increasing by 0 25 g a week to 1 5 g daily She has not had any subjective or objective negative side effects A new tracheoscopy in July 1973 showed an irregular mucous membrane but a fairly good lumen In October 1973 the mucous membrane had started to become pale and dry It was possible to reach the tracheal cannula with the bronchoscope without any difficulty At the last tracheoscopy, in January 1974, the mucous membrane was pale and the lumen normal, and the patient was consequently decannulated All medication has

now been discontinued and the tracheostoma is gradually closing spontaneously. The patient has no breathing difficulties at all

DISCUSSION

The observed healing of granulations, and the successful decannulations must in all probability be attributed to the pharmacotherapy In the first case, klorokin and penicillamine were both given from the onset Consequently, it is difficult to evaluate which of these medicines was of greater importance In case 2, however, treatment with klorokin for 3 months apparently did not influence the formation of granulation tissue Positive results were obtained only on the addition of penicillamine

It hardly seems likely that the granulation in the trachea was an immunological process No auto-immunological serum phenomena were observed in the rheumatoid factor test or in the negative antinuclear-factor test The documented, granuloma reducing capacity of the two remedies seems to be the most probable effector mechanism It can also be mentioned [illegible], that treatment with cortisone was tried [illegible] in these cases, without any success In [illegible]ast to steroids, klorokin and penicillamine have a fairly weak anti-inflammatory effect and therefore do not involve any increased tendency to infection Klorokin is a toxic remedy which, after long periods of treatment, may cause retinopathy, but with the short period of treatment in the present cases this risk is insignificant Reactions to penicillamine of the most varying kinds are common, for example thrombocytopenia, leucopenia, liver affections and reversible nephrotic syndromes and in 40 % of the patients with rheumatoid arthritis, this treatment has to be interrupted The negative side effects, however, are relatively innocuous and reversible Nor in the present cases have such side effects been observed In tracheal stenosis the dramatic, positive effect of this pharmacotherapy has demonstrated that all cases of recurrent tracheal stenosis should be simultaneously treated both operatively and pharmacotherapeutically By this means the frequency of recurrence and the suffering of the patient can be reduced to a minimum

ZUSAMMENFASSUNG

Unsere Erfahrungen bei der Behandlung von immunologischen Krankheiten mit einer Tendenz zur Bildung von Granulomata haben uns die Idee gegeben, Narbenbildungen bei sich wiederholenden Trachealstenosen pharmakotherapeutisch zu behandeln Wir haben zwei Medizinen mit verschiedenen Wirkungen kombiniert, nämlich Klorokin und Penicillamine Zwei Patienten mit sich wiederholenden Trachealstenosen wurden mit grossem Erfolg behandelt Nach vielen unerfolgreichen Operationen ist es uns jetzt gelungen, sie zu dekanylieren und zu einem normalen Leben zurückzubringen

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SALIVARY STIMULUS AND SWALLOWING REFLEX IN MAN

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Abstract The importance of receptor stimulation by saliva for the elicitation of the swallowing reflex in man was investigated by testing the capacity for repeated dry swallowing at maximum frequency in two controlled series one with stimulated and one with inhibited secretion of saliva The swallowing response was recorded manometrically A positive correlation was found between the secretion of saliva and the capacity for repeated dry swallowing. It was concluded that the swallowing reflex in man is dependent on an adequate stimulus The importance of an intact saliva secretion specially in cases with swallowing dysfunction is pointed out

With repeated swallowings at maximum frequency when no fluids were supplied (dry swallowing) the swallowing intervals successively increased and subjective swallowing difficulties arose (Månsson & Sandberg, 1974) Intact receptor function was found to be important for the ability of eliciting swallowing Decreasing stimulus due to saliva withdrawal by previous swallowings was suspected to be one limiting factor for the capacity of repeated dry swallowings Miller & Sherrington (1915) found in animal experiments that saliva was an effective stimulus for the swallowing reflex In clinical conditions of swallowing troubles and of lack of saliva little or no attention has been paid to the role of a sufficient and adequate stimulus for the swallowing reflex

The purpose of the present study was to investigate the importance of a sufficent stimulus for the elicitation of swallowing in man by studying the capacity for repeated dry swallowings at maximum frequency before and after changing the amount of salivary stimulus provided Two experimental series were performed one with stimulation and one with inhibition of the secretion of saliva

MATERIAL AND METHODS

Material

Healthy volunteers of both sexes were accepted as test subjects after a normal routine manometric examination of the pharynx and oesophagus Ten subjects took part in the series with stimulated saliva secretion and eight subjects in the series with inhibited saliva secretion The age distribution of the subjects can be seen in Figs 1 and 2

Recording

The manometric technique used has been described earlier in detail (Månsson & Sandberg, 1974) Pressures of the pharynx, the pharyngo-oesophageal sphincter, and the oesophagus were simultaneously transmitted by a nasally inserted, water filled, non perfused catheter assembly, to three external pressure transducers operating three electromanometers connected to a recorder

Repeated dry swallowing test

All subjects performed the test twice before and after the induction of change in the saliva secretory rate Each subject served as his own control Sham experiments have shown that the test is reproducible (Månsson & Sandberg, 1974)

Before testing the subject was carefully informed He was instructed to swallow eleven times as fast as possible, no fluids were given Pressure were recorded continuously during the test

The swallowing test time is defined as the time between the pharyngeal pressure peaks of the first

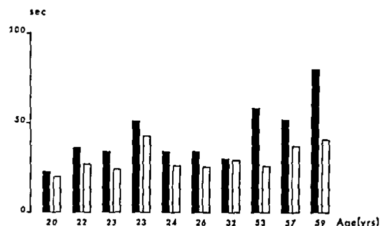


Fig. 1. Swallowing test time before (black column) and at (white column) stimulated saliva secretion. 10 volunteers.

and last swallowing of the test. *The swallowing interval* is the corresponding time between two consecutive swallowings of a test.

Stimulation of saliva secretion

During the first test the subject kept in his mouth an indifferent lozenge made of paraffin wax. During the second test an acid lozenge of the corresponding size was used in order to stimulate the secretion of saliva.[1]

The second test was started when the subject [illegible]d a feeling of increased salivation but not [illegible] than 1 minute after the intake of the acid [illegible]zenge. There was a 10 minute-pause between the two tests.

Inhibition of saliva secretion

After the first repeated dry swallowing test the 8 subjects of this group were given methyl scopolamine nitrate (Skopyl®), 0.0025 mg per kg body weight intravenously. The second test was performed 15 minutes after the injection. The subjects were instructed to report dryness in the mouth and palpitations, pulse rate was controlled.

Statistics

Sign test was used to assess differences in the swallowing test times of the groups before and after induced change in saliva secretion.

[1] Acid lozenges were kindly supplied by T. Ericson, Associate Professor. Faculty of Odontology. For their composition, see Clark et al. (1961).

RESULTS

Series with stimulated saliva secretion

Repeated dry swallowings at maximum frequency with a paraffin wax lozenge in the mouth did not differ in swallowing test time, swallowing intervals or subjectively from the corresponding test without the lozenges in an earlier study (Månsson & Sandberg, 1974). By testing with an acid lozenge in the mouth, swallowing test time decreased for all the 10 subjects (Fig. 1). Their mean decrease was 27 %. The difference is significant ($p<0.01$). The durations of the swallowing complexes were essentially unchanged, the decrease in swallowing test time was due to reduction of the times between the swallowings. The test results of one subject before and at stimulation of the secretion of saliva are shown in Figs. 3A and 4A. From Fig. 5 (left) it may be seen that the successive increase in the means of the swallowing intervals of the first test did not appear when the salivary secretion was stimulated. All subjects found the second test easier to perform.

Series with inhibited saliva secretion

After the administration of methyl scopolamine, swallowing test time increased for all 8 subjects (Fig. 2). Their mean increase was 54 %. The difference in swallowing test time was significant ($p<0.01$). The increase was due to prolonged times between the swallowing complexes. The test results of one subject before and at inhibi-

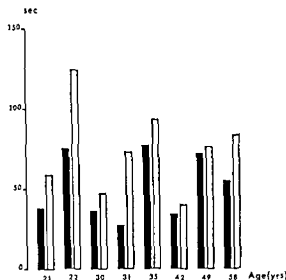


Fig 2 Swallowing test time before (black column) and at (white column) inhibited saliva secretion 8 volunteers

tion of the saliva secretion are shown in Figs 3B and 4B From Fig 5 (right) it can be seen that the increase in swallowing test time was evenly distributed amongst the means of the swallowing intervals When the secretion of saliva was inhibited, four volunteers experienced that the swallowing difficulties in the test increased, they all had an increase in swallowing test time exceeding 50%

At the start of the second test all subjects felt dryness in the mouth Pulse rate increased to 96–126 per minute, all subjects except one had slight palpitations

DISCUSSION

Swallowing test time varied from one individual to another in the first tests of both series Swallowing test time was, however, found to be reproducible in the same individual (Månsson & Sandberg, 1974) The test is suitable for studies on the swallowing reflex near the threshold level

The stimulating effect of the acid lozenge used on the saliva secretion has been shown experimentally in man (Zickert, 1974) The possible effect of having a lozenge in the mouth during the test was compensated by the use of an indifferent lozenge in the first test In animal experiments, neutral and acid water solutions were equally effective stimuli for the swallowing reflex (Storey, 1968*a*) Therefore it was presumed that the acidifying of the saliva in the present investigation did not directly affect the swallowing reflex

Methyl scopolamine is a well known quaternary anticholinergic drug which effectively inhibits the secretion of saliva even at a dosage when no other effects occur (Burgen & Emmelin, 1961, Goodman & Gilman, 1965) In the present investigation dryness of the mouth appeared and even other anticholinergic effects were noted, such as tachycardia and palpitations It is concluded that the administered dose of methyl scopolamine inhibited the secretion of saliva, central nervous effects are negligible (Goodman & Gilman 1965) Atropine has a depressive effect on the motor activity of the smooth muscle part of the oesophagus but leaves the striated muscle part essentially unaffected (Kantrowitz, Siegel & Hendrix, 1966) Methyl scopolamine is supposed to have a similar effect, but this influence on the smooth muscle part of the oesophagus is of minor importance for the present study

Mutually contrasting effects on swallowing test time were found in the two series As the secretion of saliva was affected in two different ways, it is very probable that the effect on the swallowing test time was caused by the induced changes in salivary secretion and not by other effects of the methods used to influence the secretion of saliva For the same reason it is probable that quantitative and not qualitative changes in saliva caused the effects observed on the swallowing test time

The characteristics of the repeated dry swallowing test have earlier been reported to be dependent on the sensory inflow from the oropharyngeal receptors (Månsson & Sandberg, 1974) The discharge from these receptors is correlated to the strength of the stimulus applied to them (Storey, 1968*b*) The



Fig 3 Manometric recordings of the repeated dry swallowing test of one volunteer Pressure curves of the pharynx, the pharyngo-oesophageal sphincter and the oesophagus are marked 1, 2 and 3, respectively Dotted lines represent atmospheric pressure (A) Recordings before (upper curves) and at (lower curves) stimulated saliva secretion (B) Recordings before and at inhibited saliva secretion

creased and decreased in the present investigation through stimulation and inhibition of the saliva secretion, respectively The thereby observed effects on the swallowing test time can explained by changes in the sensory inflow animal experiments with afferent nerve stimution Doty (1951) found that the swallowing eflex, like other reflexes, demands a threshold value in sensory inflow for its elicitation

In stimulation of the saliva secretion, the means of the swallowing intervals were on the whole constant during the test This could be due to either a constant stimulus being provided or a frequency limit through refractory periods This can only be proved by experiments where still more stimulus would be supplied The cause of the constant swallowing frequency seems not to lie in the effector organ, since no signs of fatigue appeared in the manometric swallowing pattern (Fig 3)

The results of the present investigation thus show that a sufficient stimulus is needed in order to elicit the swallowing reflex in man Investigations in cats also show that the stimulus must be appropriate (Miller & Sherrington, 1915) Water and saliva were effective stimuli while meat and oil were not This is one explanation of why it is easier to swallow a tablet

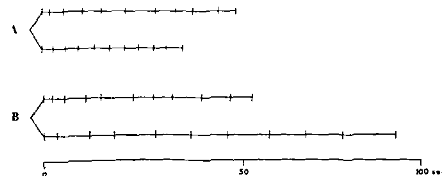


Fig 4 Diagram from Fig 3 Vertical lines represent the pharyngeal pressure peaks at swallowing

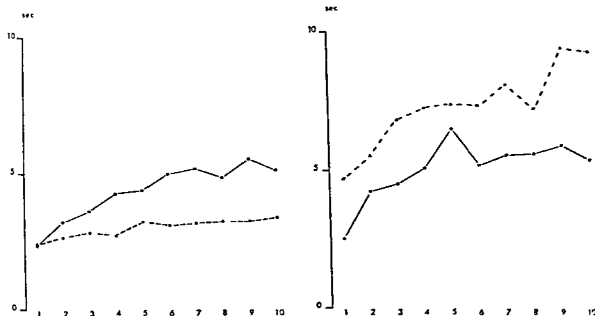


Fig 5 The means of the swallowing intervals. Arabic numerals indicate the ordinal numbers of the swallowing intervals Left series with stimulated saliva secretion, right series with inhibited saliva secretion. ●—● Before induced change in saliva secretion, ■- - -■ at stimulation of saliva secretion, ▲- - ▲ at inhibition of saliva secretion.

with water than without It also helps to explain the aspiration of oils or hydrocarbone in lipoid pneumonitis (see Olsen, 1970) An unsuitable stimulus hardly results in an afferent discharge exceeding the threshold of the swallowing reflex Thus a protective mechanism is not active, implying a risk of aspiration

As the salivary stimulus, according to the present investigation, is significant for the elicitation of the swallowing reflex, inhibition of the secretion of saliva is somewhat contra indicated in conditions of swallowing dysfunction Thus paradoxically enough, the secretion of saliva *should not be strongly restricted in patients* with chronic brain damage and long standing drooling, as swallowing disorders are frequent amongst them (Ekedahl, Månsson & Sandberg, 1974) In peripheral swallowing dysfunction, for example after partial laryngectomy, the swallowing stimulating properties of the saliva should be observed, so that irradiation will not cover salivary glands other than those the disease treatment requires

The dependence of swallowing on saliva gives rise to a suspicion that swallowing troubles could be caused by lack of saliva Defective salivary stimulus might contribute to the dysphagia that about one third of patients with Sjögren's syndrome experience (Doig et al, 1971) Among psychiatric clientele, globus is a frequent complaint One causal factor might by shortage of saliva on account of anxiety and/or drugs

ZUSAMMENFASSUNG

Die Bedeutung der Receptorstimulation des Speichels fur die Auslösung des Schluckreflexes beim Menschen wurde durch Testung des Vermögens mit schnellster Frequenz wiederholt trocken zu schlucken in zwei Gruppen untersucht Eine Gruppe mit Stimulation und die Andere mit Inhibition der Speichelsekretion Die Schluckbewegungen wurden durch Manometrie registriert Eine positive Korrelation zwischen der Speichelsekretion und des Vermögens fur wiederholtes Trockenschlucken wurde gefunden Es wurde festgestellt dass der Schluckreflex des Menschen einem ausreichenden Stimulus abhängig ist Die Bedeutung einer intakten Speichelsekretion besonders in Fällen einer Schluckdysfunktion ist hervor gehoben worden

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DIE REGENERATION DES KINDLICHEN SEPTUMKNORPELS NACH SEPTUMPLASTIKEN

Eine histologische Studie

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(Eingegangen am 15 Juni, 1974)

Abstrakt Bei 8 Kindern wurde zwischen 6,3 und 11,7 Jahren eine Septumkorrektur nach Cottle oder Goldman durchgefuhrt Sie kamen 1,2 bis 3,9 Jahre spater zur Septum Revisionsoperation, wobei aus der ehemals resezierten Basis, Mitte und Columella Kante des Septums Knorpelproben entnommen und histologisch untersucht wurden In allen Proben war eine Knorpel regeneration durch appositionelles und interstitielles Wachstum nachzuweisen am geringsten an der Resektionskante der Basis am starksten an der korrigierten Columella Kante des Septums Diese histologischen Befunde einer gewissen Regenerationspotenz operativ traumatisierten Septumknorpels des Kindes nach strukturerhaltender Septumplastik lassen sich mit den Erfahrungen aus Experimenten am wachsenden Septum von Nagern vergleichen

Aus Experimenten über partielle Entfernung von Septumknorpel bei neugeborenen oder jungen Tieren weiß man, daß bei wachsenden Nagern (Meerschweinchen, Ratten, Kaninchen) in vivo unter bestimmten Bedingungen Regenetationen des Knorpels im Resektionsgebiet beobachtet werden (Kvinnsland, 1973, Kvinnsland & Breitstein, 1973, Stenstrom & Thilander, 1972, Wexler & Sarnat, 1961) Auch in Organkulturen haben Petrovic et al (1969) die Wachstumspotenz von Chondroblasten aus dem Septumknorpel junger Ratten nachgewiesen

Von der Wachstumspotenz traumatisierten kindlichen Septumknorpels findet sich nur eine Mitteilung von Peer (1945), der bei einem 7jahrigen Kind Septumknorpel ohne Perichondrium autolog transplantierte und nach 1,5 Jahren eine Großenzunahme des Transplantats um 1,5 mm in der Länge und 0,75 mm in der Breite feststellte

Seit der Dokumentation von Jennes (1964) wissen wir ferner, daß gewebeschonende und strukturerhaltende Septumkorrekturen im Kindesalter zu keiner Wachstumshemmung der Nase führen müssen Diese Erfahrung ist von anderen Operateuren und auch an unserem Krankengut bestätigt worden (Pirsig & Knahl, 1974) Ein histologischer Beweis fur die weitere Entwicklung operierter wachsender Strukturen in der Nase beim Menschen fehlt jedoch bis heute

Ziel dieser Studie ist daher, für das wachsende Septum anhand von 8 Beispielen zu zeigen, daß eine Knorpelneubildung durch appositionelles und interstitielles Wachstum an den Resektionskanten des kindlichen Septumknorpels stattfindet

MATERIAL UND METHODE

Zur histologischen Untersuchung kamen Knorpelproben aus den Nasensepten von 8 Kindern, bei denen im Alter von 6,3 bis 11,7 Jahren wegen behinderter Nasenatmung eine Septumkorrektur nach Cottle oder Goldman durchgefuhrt worden war und die wegen erneuter Nasenobstruktion nach 1,2 bis 3,9 Jahren wieder operiert wurden (Einzelheiten Tab I)

Bei dieser Revisionsoperation fand sich als eine Ursache der Obstruktion (Pirsig &

Tabelle I *Daten über 8 Jungen, bei denen Knorpelproben aus dem Septum bei Revision-Operationen entnommen wurden*

Pat	Hist.-Nr	Alter (Jahr/Monat) bei 1 Op	2. Op	Kontroll-Intervall	Septum PE aus Basis	Mitte
S R	19/73	6/4	8 0	1/8	+	+
K. P	10/73	6/4	9 4	3/0	+	+
B J	8/73	7/0	8/8	1/8	+	+
F H	22/73	9/5	13/3	3/10		+
S M	16/73	10 3	11/5	3/2	+	+
F B	4/73	11/5	12/7	1/2	Columella Kante	
D E	12/73	11/5	13/4	1/11	+	+
K M	17/73	11/9	14/7	2/10	+	+

Knahl, 1974) ein Wachstum des Knorpels im Bereich resezierter Septumzonen wie an der Kante der korrigierten Septumbasis, im Bereich des ehemals 3–4 mm breiten vertikalen Knorpeldefekts in Septummitte oder an der getrimmten Septumkante in der Columella Aus diesen Zonen wurden kleine Knorpelstreifen im Rahmen erneuter Korrekturmaßnahmen entnommen Dabei wurde die Beziehung dieser Knorpelstückchen zum Septum dokumentiert, so daß der histologischen Aufarbeitung der in [illegible]l-Alkohol fixierten und in Paraffin eingebetteten Knorpelproben genaue topische Angaben möglich waren Bei der Gewinnung der Knorpelstücke wurde in der Regel das „äußere" Perichondrium nicht entfernt, so daß in den Präparaten nur das „innere" Perichondrium mit dem exzidierten Knorpel im Zusammenhang blieb Eine exakte Trennung zwischen Septumknorpel und inneren Perichondriumschichten ist nämlich beim kindlichen Nasenseptum präparatorisch nicht möglich Die 5–7 μ dicken Knorpelschnitte wurden mit Hämatoxylin-Eosin, Giemsa, Astrablau, Toluidinblau und PAS (Perjodsäure-Schiff-Reaktion) gefärbt und unter dem Lichtmikroskop beurteilt Als Vergleich dienten Knorpelproben aus nicht traumatisierten Septumzonen von sieben erstoperierten Jungen im Alter von 9 bis 14,7 Jahren Die genaue histochemische Auswertung des Materials und die Daten über die Struktur des normalen kindlichen Septumknorpels, speziell die Besonderheiten der Septumbasis, werden in einer weiteren Mitteilung folgen

ERGEBNISSE

Normaler Septumknorpel

Aus den nicht traumatisierten Knorpelproben des mittleren Septums der 9- bis 14jährigen lassen sich lichtmikroskopisch im Horizontalschnitt zwei polare Typen und einige Übergangsformen unterscheiden Der eine Typ (Abb 1) zeigt eine angedeutete axialsymmetrische Anordnung der Chondrocyten, die als isogenetische Gruppen im Zentrum etwa säulenförmig und senkrecht zum Faserverlauf der äußeren Perichondriumschichten orientiert sind Subperichondral sind die Längsachsen der ovalen Chondrocyten eher schräg ausgerichtet Der andere Typ weist mit Ausnahme der äußeren Perichondriumschichten keine Symmetrie im Horizontalschnitt auf Die zentralen isogenetischen Knorpelgruppen liegen ohne dominierende Richtung durcheinander Die beiderseitigen inneren Perichondriumzonen und die subperichondralen Chondrocytenzonen können unterschiedlich dick sein und zeigen meist nur eine angedeutete Gliederung ihrer Zellen

Regenerierter Septumknorpel

In den Bereichen der Resektionskanten, die vor 1,2 bis 3,9 Jahren am Septumknorpel der acht Kinder angelegt wurden, beobachtet man in jedem Fall deutlich ein appositionelles und interstitielles Knorpelwachstum Daneben finden sich Knorpelnarben in bindegewebiger oder knorpeliger Reparation und kleine Bezirke mit toten Chondrocyten ohne Gefäße Diese Bezirke sind jedoch völlig von vitalen Knorpelzellen eingeschlossen (Abb 4) Obwohl der Übergang zwischen Altknorpel und regeneriertem Knorpel meist nicht genau angegeben werden kann, läßt sich der regenerierte Knorpel an den Resektionskanten von den subperichondralen Wachstumszonen des normalen Septumknorpels

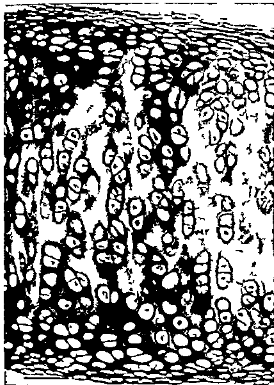


Abb 1 Horizontalschn tt aus Septummitte (Junge 10 J) Etwa symmetrischer Aufbau der beidse t gen Per chondrium u Subperichondi umzonen saulenartige An ordnung der zentralen isogenet schen Chondrocyten gruppen H st Nr 25/74 Tolu d n Maßstab 0 1 mm

(Abb 1) anhand einiger Merkmale unter scheiden

1 Im regenerierten Knorpel liegen zwischen den jungen Chondrocyten großere Areale von Knorpelgrundsubstanz (Abb 2 4) in der eine Gl ederung in Hofe Territorien und Interterri torien subperichondral oft nicht zu erkennen ist (Abb 2 oberes Drittel) Die Chondrocyten haben dann meist eine runde Form und einen kleinen Durchmesser

2 In anderen Beispielen werden die iso- genetischen Chondrocytengruppen haufig nur durch schmale Knorpelgrundsubstanzsaume voneinander getrennt und zeigen subperichondral eine großere Teilungsrate (Abb 3 5 6C) als in den subperichondralen Zonen des Altknor pels (Abb 1) Im Knorpelzentrum ahneln dabei die einzelnen isogenetischen Gruppen eher einer Traube (Abb 3 5) als einer Saule (Abb 1)

Abb 2 Regenerierter Knorpel in der Columella Kante des Septums ungerichtet gewachsen so daß e ne erneute Subluxat o sept result erte Auf der rechten B ldse te ist der Knorpel stellenweise durch d e Entnahme defoim ert H st Nr 4/73 Hamatoxyl n Eos n Maßstab 0 1 mm.

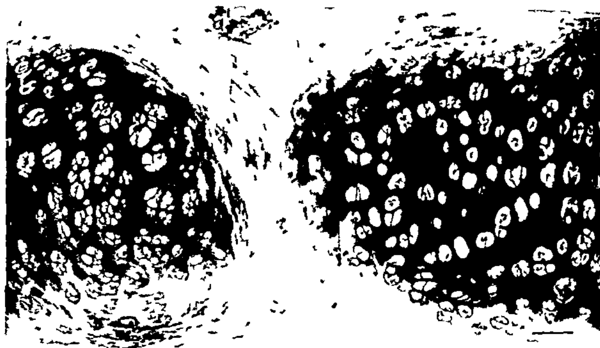


Abb 3 Horizontalsch tt dur h den vert kalen rege erier ten Knorpeldefekt in Septumm tte Mehr appos t onelle Knorpelregenerat on des rechten Defektrandes m t nur selten gete lten isogenetischen Gruppen mehr interst t elles Knorpelwachstum im l nken Defektrand m z. T zahlre chen Te lungen der traubenform gen sogen tischen Gruppen Hist Nr 17/73 c Hamatoxylin Eos n Maßstab 0 1 mm

3 Beim regenerierten Knorpel fehlen haufig dominierende Ausrichtungen oder Symmetrien einzelner Chondrocyten und der isogenetischen Gruppen innerhalb der Grundsubstanz (Abb 5) oder werden erst in der Nachbarschaft des Altknorpels erkennbar In anderen Beisp ele ist der Neuknorpel deutl ch gegliedert un erscheint als organische Verlangerung des Alt knorpels (Abb 4)

4 Einige Resektionskanten sind von eine

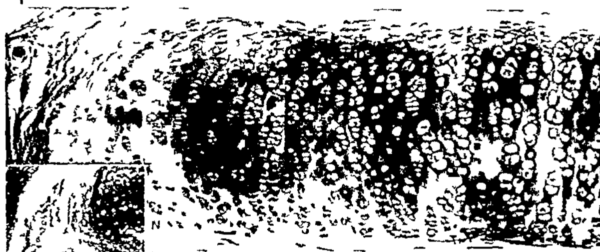


Abb 4 Hor zontalschn tt durch den vert kalen regeneri erten Knorpeldefekt in Septumm tte (Inlet Tolu d n) Im dorsalen Defektrand ist der Übergang vom Alt knorpel (rechtes B lddrittel) zum Neuknorpel unscharf Zellreichere isogenet sche Gruppen aus Chondrocyten m t kle nerem Durchmesser m Neuknorpel kontrast eren zu zellarmeren isogenet schen Gruppen aus Chondro cyten m t großerem Durchmesser Kle ne Insel m t to en Chondrocyten (N) H st Nr 19 73 Hamatoxyl n Eos n Maßstab 0 1 mm



5 Hor zontalschn tt durch den regenerierten n Defektrand aus Septummitte Kappenartig mfaßt d e B ndegewebs Perichondriumsch cht im nken B ldrand den Neuknorpel der einen größeren queren Durchmesser hat als der Altknorpel im rechten B ldrand H st Nr 17/73 Hämatoxyl n Eosin Maßstab 0 1 mm

geordneten straffen Perichondriumkappe umgeben (Abb 4 5) In anderen Proben schiebt der neue Knorpel pseudopodienartige Ausläufer in e n eher lockeres und wenig gegl edertes Bindegewebe (Abb 2 6C)

5 Benachbarte Chondrocyten konnen unterschiedlich starke Aktivitaten im Wachstum in der Teilung und in der Knorpelgrundsubstanzb ldung zeigen was s ch in der Überfarbbarkeit der Chondrocyten im HE Schnitt (Abb 2 5 6B) und in den h stochemischen Reaktionen der Grundsubstanz wiedersp egelt

Knorpel u achs

Im Bereich der basalen Septumresektionskanten ist der Übergang zwischen Altknorpel und regeneriertem Knorpel meist zu erkennen schwieriger jedoch in den Zonen der Septummitte vor allem wenn der Altknorpel zum asymmetrischen Typ gehort (Abb 5) Das ist ein Grund warum in unserem Material meist n cht die exakte Zuwachsquote fur den regenerierten Knorpel angegeben werden kann Der andere Grund ist d e oft zu kleine Knorpelprobe die kaum Altknorpel enthalt Deshalb kann nur eine semi-quantitative Aussage uber die unterschiedl che Regenerationspotenz innerhalb des Septumknorpels gemacht werden An der Septumbasis ist die Regeneration gering (z B 0 3 mm Knorpelzuwachs in 1 Jahr und 8 Monaten fur B J) im Bereich der vertikalen Resektionskanten aus Septummitte jedoch gro-

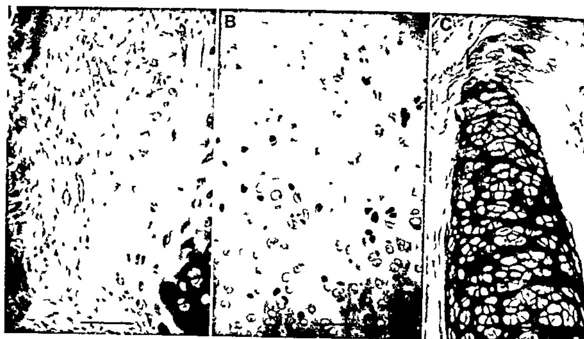


Abb 6 (Maßstab stets 0 1 mm) A Neuknorpel aus der Septumbasis mit demaskierter Grundsubstanz (oben rechts gering unten links stärker) und eindringendem Gefäß(Pfeil) Hist Nr 10/73/c Hamatoxylin Eosin B Regenerierter Knorpel aus der Columella Kante Über farbbarkeit einzelner Chondrocyten als ein Kriterium nicht synchroner Wachstumsaktivitaten. Hist Nr 4/73/b Hamatoxylin Eosin C Dorsale regener Kante aus dem vertikalen Knorpeldefekt in Sep mitte Ungerichteter Neuknorpel (oben) in g strukturiertem Perichondrium und gerichtetes s interstitielles Wachstum in Fortsetzung des Altknor Hist Nr 10/73/a Toluidin

ßer (0,9 mm Zuwachs in 1 Jahr und 8 Monaten fur S R), so daß manche Defekte bis auf einen Bindegewebsspalt von 0 15 bis 0,3 mm (Abb 3 4) zusammengewachsen waren Der großte Zuwachs an Neuknorpel findet sich an der freien Knorpelkante in der Columella (2,0–2,5 mm in 1 Jahr und 2 Monaten, Abb 2)

Bei drei Kindern (Abb 5) war der Neuknorpel an den vertikalen Resektionskanten auf Horizontalschnitten breiter im Querdurchmesser als der Altknorpel, bei zwei Kindern dagegen schmäler (Abb 6C)

Unsere Abbildungen bringen Beispiele von Knorpelregenerationen aus der Altersgruppe zwischen 8 und 14 Jahren Eine qualitative oder quantitative Altersabhangigkeit der Regeneration ergibt sich aus diesem Material nicht

Zweimal zeigen sich an der basalen Septumkante umschriebene Umbauvorgange von Jungknorpel mit Demaskierung der Grundsubstanz und einsprossendem gefäßfuhrenden Bindegewebe (Abb 6A) An der basalen Resektions kante sind auch häufiger verheilte Knor narben zu erkennen als in Septummitte I liegt zum Teil an der etwas groberen Präpa tionstechnik bei der Entfernung der hypert phen und traumatisierten Septumbasis, die nur mit einem Raspatorium aus der Verbindu mit Prämaxilla und Vomer gelost werden ka wahrend der vertikale Knorpelstreifen a Septummitte mit dem gebogenen Ritzmes schonender entnommen wird Verkalkungen d Knorpels werden an keiner Stelle beobacht

DISKUSSION

Die hier vorgelegten Ergebnisse über die Wac stumspotenz operativ traumatisierten Septu knorpels beim Kind lassen sich weitgehend n den an Nagern gewonnenen Befunden ve gleichen An neugeborenen und 7Tage alt Ratten fanden Kvinnsland & Breitstein (197 drei Monate nach Entfernung von 50% d mittleren Nasenseptumknorpels bei fast all

ieren eine völlige Regeneration des Septumnorpels Bei Ratten, die am 14 bis 28 Tag perierт waren, zeigte sich ein zunehmend ößerer Septumdefekt, dessen Ränder regene- Knorpel enthielten Wexler & Sarnat l) sahen, daß partielle submuköse Septumefekte bei 14- bis 28Tage-alten Kaninchen ach 17 Wochen durch Knorpelwachstum von 75% auf 30–40% verkleinert waren Stenröm & Thilander (1972) stellten histologisch agegen nur noch eine geringe Knorpelneuildung in den Defekten des hinteren Sepums nach Operation von 3–6 Tage alten Meerschweinchen fest Vielleicht lassen sich e unterschiedlichen Regenerationspotenzen wischen Ratten und Meerschweinchen aus den erschieden starken Wachstumsaktivitäten der hondrocyten innerhalb des Septums herleiten, ie sie fur 3Wochen-alte Kaninchen mit kiertem Thymidin nachgewiesen wurden ong et al, 1968) Auch in unserem Material aren unterschiedliche Regenerationspotenzen den einzelnen Zonen des Septums erkennbar er meiste Neuknorpel fand sich in der Columlakante und der geringste Zuwachs an der orrigierten Septumbasis, während der Zuwachs Knorpel in den Resektionskanten der norpelmitte mit 0,6 bis 1,0 mm innerhalb 5 bis 2 Jahren dazwischen lag Fuchs beob- 1932 wohl als erster lichtmikroskopisch geringe perichondrale Knorpelneubildung im Frakturspalt des Septums eines 22jährigen Mannes (Fall 16) mit Septumtrauma vor 2 Jahren Ombredanne (1942) sah dagegen bei 46 Kindern, deren Septum er im Alter von 5 bis 12 Jahren submukos nach Killian reseziert hatte, bei der klinischen Kontrolle keine deutliche Ausfullung des Resektionsbezirkes mit Knorpel

Zusammenfassend laßt sich aus diesem Schrifttum und aus den eigenen Resultaten sagen, daß beim Menschen und bei Nagern kleine Septumdefekte im jungen Alter partiell durch regenerierten Knorpel ausgefullt werden konnen und daß mit zunehmendem Alter ein Knorpelwachstum nur noch an den Defektrandern stattfindet

Da bei der Septumkorrektur nach Cottle oder Goldman nur kleine streifenförmige Septumdefekte unter Schonung des äußeren Perichondriums gesetzt werden, ist verständlich, warum wir bei einigen Kindern sogar eine weitgehende Ausfullung dieser Defekte mit neuem Knorpel bei der Revisionsoperation fanden Ein Teil der Defektausfullung ist wahrscheinlich auch durch Wachstum des ubrigen intakten Knorpels zu erklären Bei großen Septumdefekten wie nach der Killian'schen Operation reicht die Regenerationspotenz des Perichondriums zur Ausfüllung der Knorpeldefekte nicht mehr aus, wie die klinischen Ergebnisse von Ombredanne zeigen Eine vollige Überbruckung des vertikalen Defektes in Septummitte sahen wir allerdings bei keinem der Kinder Stets trennte noch ein Bindegewebsstreifen von etwa 0,2–0,3 mm Breite die beiden Knorpelkanten, die sich in zwei Fällen sogar aneinander vorbeischoben, wie bei der intraoperativen Freilegung zu sehen war Diese Defektheilung des frakturierten Septumknorpels durch fibrösen Kallus beschrieb Zuckerkandl schon 1892 beim Erwachsenen

Die Regeneration des Septumknorpels an den Defekträndern erfolgte in einigen Präparaten (Abb 3, 4) mit einer gewissen Ordnung und etwa in Fortsetzung des normalen Knorpelgefüges, so daß der Übergang zwischen Altknorpel und Neuknorpel nur unscharf zu erkennen war In mehreren Beispielen — vor allem an der Basis — war die Knorpelregeneration makroskopisch und mikroskopisch ungerichtet und fiel aus dem normalen Knorpelgefuge heraus (Abb 2, 6C) Cottle hat wiederholt auf das unberechenbare Wachstum des Knorpels im Gegensatz zum Knochen hingewiesen Sein Schuler Wexler sagte 1963 The growth potential of the septal cartilage is undetermined in any one case, but as a rule the remaining septum continues to grow and may even produce an obstruction on one side or many years later, which may need a further correction' Unsere Ergebnisse unterstreichen diese klinischen Erfahrungen und bieten eine morphologische Erklärung für Rezidiv-

tumdeviationen, nachgewachsene Knorpelleisten und erneute Subluxationen der Septumvorderkante

Die Regenerationsfähigkeit menschlichen und tierischen Septumknorpels scheint sich nicht nur auf die Nase selbst zu beschränken Hinweise für ein ortsunabhängiges Weiterwachsen autotransplantierten Septumknorpels gab erstmals Peer (1945), als er bei einem Kind Septumknorpel autolog verpflanzte und einen meßbaren Zuwachs des Transplantats nach 1,5 Jahren feststellte Ähnliche Beobachtungen machte er auch für den autotransplantierten Ohr- und Rippenknorpel beim Kind Tierexperimentell hat Kvinnsland (1973) das ortsunabhängige Wachstumspotential autotransplantierten Septumknorpels bei der jungen Ratte belegt

Die Regeneration des kindlichen Septumknorpels an den Resektionskanten geschieht durch appositionelles Wachstum aus dem verbliebenen Perichondrium und durch interstitielles Wachstum Der Reifungsprozeß des Knorpelblastems verläuft zeitlich jedoch nicht immer synchron, wenn man histochemische Reaktionen (Perjodatreaktivität, Metachromasie, Basophilie) als Kriterien nimmt Dieses Nebeneinander verschiedener Differenzierungs stadien des Knorpels ein und desselben Skelett stückes wurde auch tierexperimentell (Graumann, 1964, Hommerich & Flemming, 1969, Long et al, 1968) beschrieben Eine andere Besonderheit des regenerierten Knorpels ist die Häufung von zellreichen isogenetischen Knorpelzellgruppen unmittelbar unter dem Perichondrium Die vergleichbaren Gruppen trifft man beim „normalen" Knorpel meist nur im Zentrum des Knorpels (Abb 1) Andererseits finden sich Zonen unter dem Perichondrium, in denen man nach isogenetischen Gruppen aus mehr als vier Chondrocyten vergeblich sucht Statt dessen liegen isolierte oder zweigeteilte Chondrocyten in größeren Arealen von Knorpelgrundsubstanz in Abständen voneinander getrennt, die das Mehrfache ihrer Durchmesser betragen können (Abb 2 4, 6B)

Überraschend sind die nur wenigen Hinweise auf Knorpelum- und abbauprozesse im Bereich der traumatisierten Zonen Nur zweimal fa[illegible] sich ein Bezirk mit deutlicher Grundsubstan[illegible] demaskierung und Abbau von jungem Knorp[illegible] durch einsprossendes Granulationsgewebe Zw[illegible] mal sah man kleine Einschlüsse von toten Kno[illegible] pelzellen, die völlig von vitalem Knorpel u[illegible] hüllt waren Verkalkungen des Knorpels war[illegible] nicht zu beobachten

Abschließend kann aus diesen Ergebniss[illegible] gefolgert werden, daß die Sorge vor ein[illegible] Wachstumshemmung des kindlichen Septu[illegible] nach strukturerhaltenden Septumkorrektur[illegible] unter Schonung des äußeren Perichondriu[illegible] wohl nicht begründet ist

DANK

Herrn Prof Dr J Lindner, Abteilungsdirektor [illegible] *Pathologischen Institut der Universität Hambu[illegible]* danke ich herzlich für Anregungen bei der Interpretati[illegible] der Befunde Fräulein I Lehmann gilt mein Dank f[illegible] ihre unermüdliche technische und photographisc[illegible] Assistenz

SUMMARY

In eight boys aged 6 3 to 11 7 years a septoplasty h[illegible] been performed according to the technique of eith[illegible] Cottle or Goldman Because of new nasal obstruction [illegible] to 3 9 years later a second septoplasty was carried o[illegible] In this operation, little pieces of cartilage were excis[illegible] from the formerly resected base middle and columel[illegible] border of the septum for histological examination [illegible] all pieces a regeneration of the septal cartilage [illegible] [illegible] and [illegible] growth could be found

[illegible]

histological findings of a regenerative potential of sur[illegible] cally traumatized septal cartilage in children by co[illegible] servative septoplasty can be compared with the expe[illegible] ences in the growing septum of rodents

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PENETRATION OF AZIDOCILLIN INTO THE SECRETION AND TISSUES IN CHRONIC MAXILLARY SINUSITIS AND TONSILLITIS

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Abstract The penetration of azidocillin into the sinus mucosa and secretion as well as into tonsils and adenoids was studied Altogether 73 patients were included and they were arranged in four groups according to age, diagnosis and drug dose The average azidocillin con centration was satisfactory and predictable in all tissues and well over the MIC for most upper respiratory tract pathogens studied In the maxillary antrum secretion the values were much lower and in many cases under the MIC for hemophilus This is thought to depend upon the drug s being bound to proteins in the secretions The *clinical results were good in all cases and the contribution* of the surgical operation particularly in providing drainage for the secretion is obvious

Despite the use of antibiotics, paranasal sinus infections still cause clinical problems One of e factors contributing to therapeutic failures obviously an insufficient penetration of the ntibiotic into the sinus mucosa and secretion That is why Lundberg et al (*1969*) recommend the use of large doses of penicillin derivatives to achieve adequate antibiotic concentrations in the sinus secretions On the other hand, penicillin penetrates well into lymphoid tissue, and Rasmussen (1969) found the same concentrations of benzylpenicillin in the tonsils of 15 patients with chronic tonsillitis as in normal tonsils

To evaluate the penetration of azidocillin into tissues, and its dependence upon the serum concentration, we have made a clinical study on patients with chronic maxillary sinusitis and tonsillitis Both the serum and tissue concentrations were measured and the clinical results evaluated

MATERIAL AND METHODS

This study was carried out during 1973 in the University Central Hospital of Oulu on 73 patients with symptoms of chronic maxillary sinusitis, or of chronic tonsillitis The patients were distributed into four groups as shown in Table I Those with chronic sinusitis were operated on with the radical maxillary antrum operation according to Caldwell Luc 19 operations were unilateral and 14 bilateral All tonsillectomies were performed by dissection and adenotomies by adenoid curettage and punches

Azidocillin dosage

All azidocillin doses were given intramuscularly *The drug dosages given to the four test groups* on the day before the operation and postoperatively are shown in Table I The dose was divided into two for groups A to C and into three for group D On the day of operation the dose, given 2–2 5 hrs before the operation was 0 75 g, 1 5 g, 0 75 g and 50 mg/kg respectively, in each group

Determination of drug concentrations

Blood specimens were drawn immediately before the first administration of the drug (zero sample) at the time of operation and at 0 5, 1, 2 3 4 5 and 8 hrs after the operation Samples of mucosa and secretion were collected during the operation All secretion samples with visible

Table I *The test groups and azidocillin doses*

Group	Number of patients	Age	Diagnosis	Azidocillin dose
A	21	Adult (23–68 years)	Chronic max. sinusitis	1 5 g day
B	12	Adult (19–65 years)	Chronic max sinusitis	3 g day
C	23	Adult (20–46 years)	Chronic tonsillitis	1 5 g day
D	17	Children (3 11 years)	Chronic tonsillitis	50 mg kg day

blood were discarded From patients of groups C and D both tonsils and adenoids were collected for azidocillin concentration determinations All samples were stored at −25°C until the assays were performed The tissues were homogenized by all glass tissue grinders, and 0 01 ml of undiluted tissue fluid was pipetted on to the filter paper discs

Microbiological assay

Azidocillin concentrations were determined by the microdisc method designed by Jalling et al (1972) Sarcina lutea ATCC was used as the test organism

Bacteriological analyses

Bacteriological examination was performed on secretion samples obtained from sinuses pre operatively by puncture, during operation, and

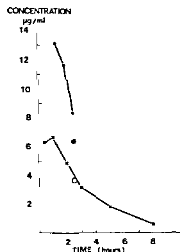


Fig 1 Azidocillin concentrations in serum (● ● 3 g day dose 1 5 g day dose) and in mucosa (● 3 g day dose ○ 1 5 g day dose)

postoperatively by irrigation Bacteriological cultivations were made on the following media blood agar, chocolate agar, lactose agar plate and thioglycolate broth

RESULTS

Maxillary sinusitis

The study on chronic maxillary sinusitis was made with two different azidocillin doses, 0 75 and 1 5 g on the morning of the day of surgery 33 patients with chronic maxillary sinusitis were operated

Serum concentrations are seen in Fig 1 With the double dose of azidocillin, the concentra tions in the serum up to 3 hrs were nearly duplicate

Penetration of azidocillin, with these doses, into the mucosa and into the secretion of the maxillary sinus are seen in Tables II and III One and a half hours after the drug was administered, azidocillin concentrations in the mucosa generally exceed the average minimum inhibitory concentration (MIC) of this drug for most clinically important bacteria (Forsgren, 1969) One hour later the concentrations in serum and mucosa were approximately equal The concentration figures obtained with the higher dose are also higher in the mucosa but in the secretion they do not appreciably differ from those obtained with the lower dose Penetration of azidocillin into the maxillary sinus secretion varied but in several instances the concentrations with both doses exceeded the average MIC values for most acute upper respiratory tract pathogenic bacteria All patients with chronic maxillary sinusi symptom-free at postoperative ch

Table II *Azidocillin penetration into maxillary sinuses (Dose 1 5 g/day)*

Patient	Side of operation	Azidocillin concentr in mucosa	Azidocillin concentr in secretion	Bacterial findings before treatment	Bacterial findings at time of operation	Post operative irrigation
1 P K.	dx	3 51	—	*H influen ae*	neg	neg.
	sin	5 76	—			
2 P S	sin	0 91	1 08	neg	neg	neg
3 E M	dx	6 78	0 67	neg	neg	neg
	sin	4 65	—			
4 V J	dx	1 00	1 00	*E coli*	neg	neg.
5 V K	dx	7 14	—	*H influen ae*	*H influen ae*	neg.
	sin	2.38				
6 L H	dx	1 48	—	neg	neg	neg
7 R A	dx	9 09	0 40	neg	neg	neg.
	sin	9 52	0 16			
8 E P	sin	—	0 16	neg	neg	neg
9 T T	dx	6 69	1 00	neg	neg	neg.
10 R P	dx	—	0 77	neg	neg	neg
11 A L P	sin	7 14	—	*H influen ae*	neg	No secretion
12 V M	dx	1 91	—	neg	neg	neg
13 V T	dx	5 41	1 82	*Staph aureus*	neg	neg.
14 M M	sin	3 33	1 25	*H influenzae*	neg	neg
15 P M	dx	6 35	0 32	Enterobacter	neg	neg.
	sin	6 9	0 44		No secretion	No secretion
16 Y L.	dx	0 89	0 67	*S aureus*	neg	neg
17 E V	dx	4 65	0 38	*D pneumoniae*	*D pneumoniae*	neg
	sin	1 82	—			
18 U V	sin	0 44	—	*H influenzae*	neg	neg
19 H T	sin	6 35	—	*K ozeanae* *S aureus*	neg	neg
20 J H	dx	1 29	0 37	neg	neg	neg
21 S R	sin	0 56	—	neg	neg	neg
		Mean 3 60 Range (0 44–9 52)	Range (0 16–1 82)			

docillin treatment having been continued for 4 days

Chronic tonsillitis

The study on chronic tonsillitis was made with only one azidocillin dose (1 5 g/day for adults and 50 mg/kg/day for children) 23 adults and 17 children were operated The antibiotic concentration data in tonsils adenoids and serum are seen in Table IV Even 1½ hrs after the drug was injected the azidocillin concentrations exceeded the clinically most important MIC values The concentration figures for the tonsils and adenoids of children are in the same range as for adults The postoperative antibiotic course was 7 days and all these patients were also symptom free a fortnight later

No side effects were noted in any group during the medical treatment, but all persons known to be allergic to penicillin were excluded from the azidocillin treatment

DISCUSSION

Seventy three patients with symptoms of chronic maxillary sinusitis and chronic tonsillitis were operated and azidocillin concentrations were determined in serum, tissues and secretions Our work confirms earlier studies (Lundberg et al , 1969, Rasmussen, 1969, Lundberg 1974) concerning antibiotic penetration into the sinus mucosa and into secretion, and into the tonsils

Azidocillin concentration in the sinus mucosa followed the serum concentrations closely (Tables II and III) From the clinical point of view this is important because the actual site of infection is in the mucosa, sinus secretion being

Table III *Azidocillin penetration into maxillary sinuses (Dose 3 g/day)*

Patient	Side of operation	Azidocillin concentr in mucosa	Azidocillin concentr in secretion	Bacteriological findings before treatment	Bacteriological findings at time of operation	Post operative irrigation
1 H P	dx	5 88	—	Proteus sp	neg	neg
	sin	4 94	—	*Streptococcus β hemolyticus* group A		
2 E. M	dx	5 00	—	neg	neg	neg
3 A. K	sin	6 06	0 16	*S aureus*	neg	neg
4 M P	dx	7 14	—	neg	neg	neg
	sin	9 52	—			
5 A K A	sin	3 64	0 80	*H influen ae*	neg	neg
6 Y N	dx	7 27	0 16	*H influen-ae*	neg	neg.
	sin	6 45	—			
7 T L.	dx	5 71	1 70 (cyst)	neg	neg	neg
	sin	5 00	—			
8 S A O	dx	3 85	—	neg	neg.	neg.
9 I P O	dx	7 14	0 16	*S aureus*	neg.	neg
	sin	5 15	—			
10 A O	sin	4 00	—	*D pneumoniae*	neg	neg
11 T K	dx	11 11	0 77	*Proteus mirabilis*	neg	neg.
	sin	8 89	0 16			
12. E P	dx	5 88	0 16	*S aureus*	neg	neg
	sin	8 00	0 33			
	Mean	6 35				
	Range	(3 64–11 11)	Range (0 16–0 80)			

a rich substrate in which bacteria may live and multiply Therefore, the removal of pathologically changed mucosa and the opening of a good drainage for sinus secretions is the rationale of a radical maxillary operation The contribution of this operation to a good clinical result is obvious Further evidence for this is the fact that in the sinus secretion of at least 4 of our patients there were gram negative bacteria resistant to azidocillin (Tables II and III), and all these patients were postoperatively symptom free

Table IV *Azidocillin concentration in tonsils and adenoids*

	In tonsils	In adenoids	In serum
Adult	1 30 (0 16–4 17)	—	4 76 (1 78–5 00)
Children	1 40 (0 45–4 00)	1.26 (0 29–3 70)	3 63 (1 78–5 12)

The mean values are calculated from concentration data obtained during the first 2 hrs after azidocillin administration Ranges are shown in parentheses

It appears, from the data shown in Tables II, III and Fig 1, that the azidocillin concentration in secretions does not parallel the blood and mucosa concentrations The administration of a double dose of the drug did not result in a proportionate increase in the sinus secretion In fact, the concentrations were as low as in the secretions of group A patients Lundberg et al (1969) reported the same finding with benzylpenicillin, the concentration of which was always lower in the secretion than in the mucosa and did not parallel the drug dose The variation in the secretion concentrations of azidocillin, irrespective of the corresponding values in mucosa, may be due to the binding of azidocillin with proteins of varying amounts in the sinus secretion (Verwey et al, 1966, Rolinson & Sutherland, 1965, Kunin, 1967, Lundberg, 1974)

Azidocillin in concentrations high enough to kill hemophilus (MIC 0 63, Forsgren, 1969) was found in only a few secretions This may explain the finding that one hemophilus was cultured in the secretion even at a

operation However, the clinical result was good in all these cases This gives further weight to the statement that, for good clinical results, a good drainage is necessary in addition to the medical treatment Therefore, an active treatment of the sinusitis with weekly antral punctures, possibly with a medial infraction of the middle turbinate, is the method of choice for removing the nutrient secretion from the sinus and for helping the return of the normal drainage mechanism If the use of antibiotics during the first two weeks combined with weekly antral irrigations does not result in a cure within 6–7 weeks, a Caldwell Luc procedure is indicated It is then obvious that even if there are sufficiently high concentrations of the antibiotic in the mucosa during treatment, the bacteria living in the secretion may re infect the mucosa as soon as the drug is discontinued

The concentrations in the mucosa were well above the azidocillin MIC range for hemophilus That one pneumococcus was also cultured at the time of operation may be due to the short preoperative medical treatment As to other bacteria the concentrations in secretions and tonsils were in the range of or well above, the average azidocillin MIC for such important [illegible]l pathogens as group A Streptococci, [illegible] cocci and *Staphylococcus aureus* (not [illegible] penicillinase) Their respective MIC values are 0 004 0 008 and 0 03 (Forsgren, 1969)

According to Palva T et al (1962) the bacteria most frequently cultured in chronic maxillary sinusitis are *Hemophilus influenzae* (11 %), *Diplococcus pneumoniae* (20 %) and *Algaligenes faecalis* (8 %) In the large series (241 patients) of Kortekangas (1964) concerning cases of acute and subacute maxillary sinusitis the most frequently isolated organisms were *D pneumoniae* (43 %) and *H influenzae* (22 %) In our material (Tables II and III) *Hemophilus influenzae* was found in 24 % *S aureus* in 18 % and *D pneumoniae* in 6 % of the cases In 35 % of the cases the culture was negative

According to our results azidocillin is effective in acute tonsillitis Our chronic material included two patients with low tonsil concentrations (0 16 μg/ml), probably due to tissue fibrosis caused by chronic infection In cases like these no medical treatment is any longer effective and tonsillectomy is indicated The present results suggest that azidocillin can be used effectively in both acute and chronic maxillary sinusitis and tonsillitis A daily dose of 1 5 g (adults) and 50 mg/kg (children) is sufficient for a satisfactory clinical result From neither a bacteriological nor a clinical point of view is a higher dose motivated

ZUSAMMENFASSUNG

Untersucht wurde das Eindringen von Azidocillin in den Sinus mucosa und in die Sinus Sekretionen sowie auch in Tonsillen und Rachenmandeln Die Versuchsreihe umfasste insgesamt 73 Patienten die nach Alter, Diagnose und Arzneidosis in vier Gruppen eingeteilt wurden In allen Geweben war die durchschnittliche Azidocillinkonzentration befriedigend und voraussagbar *und lag uber dem MIC fur die meisten Pathogenen der* oberen Atemwege In den Sinus Sekretionen waren die Werte bedeutend niedriger und lagen in vielen Fallen unter dem MIC fur Hamophilus Es wird vermutet dass dies von dem Medikament abhängt das sich in den Sekretionen mit Proteinen bindet Die klinischen Resultate waren in allen Fallen gut und die zusätzliche Besserung durch die Operation die vor allem fur einen Abfluss der Sekretion sorgte ist offensichtlich

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TREATMENT OF ACUTE MAXILLARY SINUSITIS

IV. *Ampicillin, Cephradine and Erythromycinestolate with and without Irrigation*

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Abstract Six groups, eac ontaining 50 patients with acute maxillary sinusitis, were treated with ampicillin plus nasal decongestant, ampicillin plus irrigation, cephradine plus nasal decongestant, cephradine plus irrigation, erythromycinestolate plus nasal decongestant, erythromycinestolate plus irrigation The diagnosis was radiologically established and the healing likewise radiologically assessed on the fifth, tenth and fifteenth day Treatment was given for 10 days All groups demonstrated a similar radiological healing except cephradine plus nasal decongestant which was inferior to the others Contrarily, side effects were least frequent in the cephradine groups and most frequent in patients cured with ampicillin The difficulty in choosing the best treatment is discussed in relation to such factors as therapeutic results side effects long term consequences of antibiotic ..., establishment of any bacterial etiology and ...ion characteristics of antibiotics into the diseased

In previous reports, thirteen different modes of treatment for acute maxillary sinusitis have been compared using radiological examinations (Axelsson et al, 1970*a* 1971 1973) Drainage measures only, peroral treatment with antibiotics or these antibiotics combined with irrigation in general afforded similar treatment results Only two therapeutic measures were shown to be inferior, i e nasal decongestant only and high doses of penicillin V combined with a nasal decongestant The antibiotics tested were low and high doses of penicillin V, lincomycin, methacycline, doxycycline and spiramycin The present investigation aims at comparing three further antibiotics, i e ampicillin, cephradine, a new cephalosporin, and erythromycinestolate Satisfactory results with ampicillin have been reported in the treatment of sinusitis (Lake, 1971) Satisfactory results with cephalosporines for the treatment of upper respiratory infections including sinusitis have also been reported (Gomez & Gomez, 1970, Murphy, 1971), and similarly with erythromycinestolate (Calesnick, 1960, Herrell et al, 1960, Grainger, 1969) Björkvall (1967) used erythromycin locally in the diseased sinus on a large population of sinusitis with excellent results In comparison with a small patient group which was only irrigated, Björkvall demonstrated that the number of irrigations needed diminished significantly when erythromycin was used locally

MATERIAL AND METHOD

Patients who attended the ENT department because of clinical symptoms or signs of sinusitis were sent for radiological examination The method for the demonstration of sinusitis radiologically has been described previously (Axelsson et al, 1970*b*, Chidekel et al, 1970)

The present investigation included only patients with acute maxillary sinusitis with secretion The patients with a confirmed radiological diagnosis were consecutively allocated to one of the following treatment regimes

A *Ampicillin + nasal decongestant*

Patients were treated orally with capsules of ampicillin (Pentrexyl®, Bristol) 0 5 g three times

aily for 10 days plus nasal decongestant, xymethazolinechloride (Nezeril®, Draco), three drops in each nostril three times daily for 10 days

B *Ampicillin + irrigation*

Patients were treated with ampicillin as in group A and were irrigated every second day until the returned irrigation liquid was clear

C *Cephradine + nasal decongestant*

Patients were treated orally with capsules of cephradine (Velosef®, Squibb) 0 25 g, four times daily for 10 days plus nasal decongestant as in group A

D *Cephradine + irrigation*

Patients were treated with cephradine as in group C and were irrigated as in group B

E *Erythromycinestolate + nasal decongestant*

Patients were treated orally with tablets of erythromycinestolate (Ilosone®, Lilly) 0 5 g twice daily for 10 days plus nasal decongestant as in group A

F *Erythromycinestolate + irrigation*

Patients were treated with erythromycinestolate as in group E and were irrigated as in group B

The radiological healing was assessed on day five, ten and fifteen The radiological examination in the irrigation groups was always done before irrigation to reduce the risk of confusing the irrigation liquid with sinus secretion The radiological sinus changes were graded according to the following six point scale below

No changes	0 points
Mucous membrane thickening <6 mm	1 point
Mucous membrane thickening >6 mm	2 points
Secretion	2 points
Mucous membrane thickening <6 mm + secretion	3 points
Mucous membrane thickening >6 mm secretion	4 points
Complete opacity	6 points

The material was analysed by conventional methods including analysis of variance and covariance analysis The level of significance used in all tests was 95% The material was analysed by computer technique, OSIRIS system, which besides the treatment result also assessed the concentration of antibiotic in sinus secretion and several other parameters The latter results will be reported separately

The present material comprises 299 patients with acute maxillary sinusitis analysed initially (Table I) In all 116 patients with 139 initially completely opaque maxillary sinuses, a single diagnostic irrigation was performed to demonstrate secretion The distribution of initially completely opaque sinuses in the six treatment groups is seen in Table I In 45 patients, the frontal sinuses were affected as well but these were not assessed during healing In 36 patients, the contralateral maxillary sinus demonstrated mucous membrane thickening but without secretion and these sinuses were also regarded In six contralateral sinuses without initial radiological changes, the subsequent radiological examination demonstrated a deterioration and these sinuses were included

RESULTS

There were no statistical differences in radiological states between the groups initially Further, the different treatment groups are comparable with respect to sex and age distribution as well as the number of unilateral and bilateral changes, the number of affected frontal sinuses and the number of completely and not completely opaque sinuses (Table I) All treatment groups demonstrated progressive healing during the treatment (Table II, Fig 1) There were no statistical differences in the radiological state between the groups on the fifth and fifteenth day The group treated with cephradine and nasal decongestant was statistically significantly less improved than the two groups treated with ampicillin + irrigation and erythromycinestolate + irrigation after 10 days treatment (P 95) There was also a tendency for the cephradine + nasal decongestant group to have improved less than the other groups on the fifth and fifteenth

Table I *Acute maxillary sinusitis Present material Number of patients and diseased sinuses*

	Ampicillin nasal decon gestant	Ampicillin + irrigation	Cephradine + nasal decon gestant	Cephradine + irrigation	Erythromycin estolate + nasal decon gestant	Erythromycin-estolate + irrigation	Tot
Women	33	31	33	30	32	32	191
Men	17	19	17	19	18	18	108
Total	50	50	50	49	50	50	299
Mean age years	33	34	34	36	31	36	204
Sinuses analysed initially	81	79	78	74	75	71	458
Sinuses analysed after 5 days	76	77	74	73	75	66	442
Sinuses analysed after 10 days	69	67	69	68	62	65	400
Sinuses analysed after 15 days	80	74	76	72	72	68	442
One sinus affected	18	17	20	20	22	27	124
Both sinuses affected	27	23	22	23	17	18	130
Frontal sinus also affected	5	10	8	6	11	5	45
Not completely opaque sinuses	56	58	58	46	58	43	319
Completely opaque sinuses	25	21	20	28	17	28	139

day The groups treated with irrigation were always more improved than those treated with nasal decongestant and the respective antibiotic There was a tendency for the groups treated with ampicillin + irrigation and erythromycinestolate + irrigation to be better radiologically the other groups after 5 days of treatment

The side effects are noted in Table III In almost all instances they were mild and could often be attributed to the disease as well as to the treatment In two cases, the treatment with erythromycinestolate had to be stopped becau of side effects The most frequently reporte reactions were loose bowel movements and u specific fatigue

When the present material was divided int three groups with unilateral changes or bilater maxillary changes or bilateral maxillary an frontal changes, there was initially a pr nounced difference between the groups Ur lateral maxillary changes were more marked th the other two On the fifth day, the unilater

Table II *Acute maxillary sinusitis Radiological gradation[a] of all maxillary sinuses before and dur treatment*

	Before treatment	Mean number of points/sinus			% completely cured
		5 days	10 days	15 days	
Ampicillin + nasal decongestant	4 12	2 99	1 73	1 05	40
Ampicillin + irrigation	4 03	2.49	1 36	0 99	46
Cephradine + nasal decongestant	3 95	3 30	2 46	1 62	28
Cephradine + irrigation	4 26	2 99	1 85	1 13	33
Erythromycinestolate + nasal decongestant	3 96	3 05	1 81	1 22	28
Erythromycinestolate + irrigation	4 32	2 62	1 57	1 13	41

[a] The point scale adopted is given on page 467

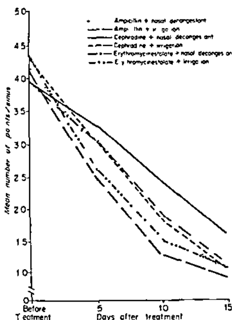


Fig 1

changes had improved most The difference between the groups was completely absent on the fifth, tenth, and fifteenth day This indicates that apart from a rapid initial healing of unilateral sinusitis, both unilateral and bilateral sinusitis heal similarly during treatment and, further, that the simultaneous involvement of the frontal sinuses does not prolong healing

DISCUSSION

The logical treatment of acute maxillary sinusitis, an infection situated in a cavity surrounded more or less by bone, should consist of restoration of drainage and antibacterial treatment Both of these therapeutical measures have well known advantages and disadvantages and/or it is by no means evident which method is preferable This in turn, is also due to a lack of appropriate methods with which to measure healing in sinusitis The patient's subjective impression, or the nasal status, or the appearance of the irrigation liquid are obviously too uncertain to give reliable information on the disease course

The ideal antibiotic would be the most effective one against bacteria most frequently demonstrated in the diseased sinus However, the knowledge available showing the bacterial flora in the aspirated sinus secretion has often shown conflicting findings (Urdal & Berdal, 1949, Palva et al, 1962, Lystad, et al, 1964, Burdin et al, 1965, Kessler, 1968, Brandt et al, 1969, Krajina et al, 1969, Axelsson & Brorson, 1972, 1973*a*) Furthermore, in general, nasal samples have been held to indicate the sinus flora which, however, does not reflect the sinus flora correctly (Axelsson & Brorson, 1973*a*) Even when aspirated sinus secretion is obtained

Table III *Side effects*

	Ampicillin + nasal decongestant	Ampicillin + irrigation	Cephradine + nasal decongestant	Cephradine + irrigation	Erythromycin estolate + nasal decongestant	Erythromycin-estolate + irrigation
Fatique, vertigo	8	7	6	8	8	7
Anorexia, nausea vomiting		1	1	1	1	
Loose bowel movements[a] and/or abdominal pain	11	11	1	2	3	7
Intra hepatic cholestatic jaundice						1 (1)[b]
Deterioration of gastritis			1			
Obstipation		1			1	1
Epistaxis after irrigation		1		1		1
Leg cramps					1	1
Vaginal fluor and/or genital itching	1		2	1		1 (1)
Exanthema		1				
Number of patients	17	17	10	9	12	17

[a] More than one loose defecation on one or more days
[b] Figures within parentheses indicate the number of patients in whom treatment was interrupted beca

for bacteriological examination, the sampling technique, the transport time and aerobic conditions influencing anaerobic bacteria unfavorably are some of the factors which might distort a correct bacteriological examination result This clearly demonstrates that bacteriological measurements are inappropriate for the measuring of healing in sinusitis

Another approach when choosing an antibiotic is comparison of the ability of different antibiotics to penetrate the sinus fluid Not many investigations have, however, as yet been performed (Lundberg et al, 1968, 1969, Gullers et al, 1969, Gnarpe & Lundberg, 1971, Lundberg & Malmborg, 1971, Jeppesen & Illum, 1972, Axelsson & Brorson, 1973b, 1974) Furthermore, the concentration of antibiotics in sinus secretion gives no information on the course of healing

In the present investigation, the therapeutic outcome was assessed by sinus radiology during the course of healing This method was adopted because of the above described lack of other reliable, appropriate methods of measuring healing in sinusitis The advantages of the radiological examination are the reproducibility and e accuracy of the method in reflecting the ' topathological sinus changes All the infectious organisms causing acute maxillary sinusitis induce the same histopathological changes with mucous membrane thickening and secretion which can both be demonstrated by radiological examination

When dealing with the assessment of the cure of a disease, it is obviously important to define the disease concept In the present investigation, acute maxillary sinusitis is defined as radiological maxillary changes with secretion in patients with clinical symptoms indicating acute sinusitis Secretion was measured because it reflects a severe impairment of the sinus mucosa and the ostial function Allergic or vasomotor disorders might have been included in the present material also afflicting the sinus mucosa since they are difficult to exclude with certainty in the individual case Furthermore the secretion in the maxillary sinus of allergic or vasomotor origin is a favorable medium for growth of bacteria even in cases which are primarily non infective which is a common experience

The single initial diagnostic irrigation of th completely opaque maxillary sinuses to demonstrate secretion may have influenced healing The distribution, however, of completely opaqu maxillary sinuses was fairly even among th groups

The result of the treatment in the presen investigation shows that all groups healed similarly favorably except the group treated with cephradine + nasal decongestant This may b because the cephradine dosage was too low an because of low bone tissue concentration (Neiss, 1973) To achieve a comparable treatment result with cephradine as with other therapeutic measures of the present and previou investigations, this antibiotic has to be combined with irrigation On the other hand, in treatmen with ampicillin or erythromycinestolate the addition of irrigation did not further improve the healing course significantly The inferior result with cephradine was also reflected by comparatively low antibiotic concentrations in sinus secretion (Axelsson & Brorson, 1974) It appears from previous investigations that the most common antibiotics are effective against the most common agents causing acute maxillary sinusitis Consequently, in the choice between different antibiotics, other factors than the bacteriological finding and the bacterial resistance pattern must also be considered These include the occurrence and severity of side effects, the concentration of antibiotic in sinus secretion the cost of treatment, etc From the patient's point of view, it is preferable to receive an antibiotic which is easy to take and requires fewer visits to the physician On the other hand, the widespread use and misuse of antibiotics must be diminished because of the increasing frequency of resistant strains, etc It can also be argued that the irrigation has less influence on the organism than has the antibiotic treatment One also has to consider the cost of therapy—in the one case, of antibiotic treatment with a higher medicine cost and in the other case, of irrigation with a higher treatment cost With

·d to all these factors, it is difficult to choose ›ptimal treatment

the present investigation, the best treatment ts were achieved, in order, by ampicillin, ıromycinestolate, and cephradine Contrari- ıe frequency and severity of side effects was ›pposite Reports on the frequency and side ts in general show a great variety of results there are reasons for believing that the re- ed incidences of side effects in general are The reported frequency is dependent upon mode of questioning the patient, varying ı a mere mentioning of side effects reported ıtaneously by the patient, to a detailed tionnaire of possible adverse effects in the vidual case In the present investigation, pa- s were asked about untoward reactions dur- and after completed treatment in the sense you experience any untoward reactions the therapy All answers are included in le III In most cases, the side effects were ırded as mild and the treatment did not need e stopped As expected, the most frequent tions were gastrointestinal The rather high urrence of fatique and vertigo can be ex- ıned by the disease per se or by the antibiotic tment

e lack of controlled clinical studies of sinusi- is well known (Catlin et al, 1961) The anti- tics tested and compared in the present in- tigation are probably often used in clinical ctice and the literature contains some state- nts which generally reflect positive experien- with these antibiotics However, no con- lled studies in sinusitis have been found in the rature The different treatment modes tested d compared in the present controlled study n all be recommended, except cephradine + sal decongestant, which had inferior results to e other groups but relatively few side effects he other five remedies achieved rapid healing ith a moderate frequency of side effects It is bvious that there is need for controlled studies f the "everyday" diseases which from the ientific viewpoint seem unattractive but which ccording to their high frequency have important onsequences

ZUSAMMENFASSUNG

Sechs Gruppen bestehend aus je 50 Patienten mit akuter Maxillarsinusitis wurden mit Ampicillin und Nasen tropfen, Ampicillin und Kieferhohlenpunktion Cephadrine und Nasentropfen, Cephadrine und Kieferhohlenpunktion, Erythromycinestolate und Nasentropfen sowie mit Erythromycinestolate und Kieferhohlenpunktion be handelt Die Diagnose wurde radiologisch verifiziert, und die Heilung wurde ebenfalls radiologisch kontrolliert, und zwar am funften, zehnten und funfzehnten Tag Die Behandlung erstreckte sich uber zehn Tage

Alle Gruppen zeigten radiologisch einen ahnlichen Heilungsverlauf mit Ausnahme der Gruppe, die mit Cephadrine und Nasentropfen behandelt wurde Diese zeigte weniger gute Resultate Dagegen waren Nebenerscheinungen bei Behandlung mit Cephadrine seltener und bei der Ampicillinbehandlung haufiger Die Schwierigkeiten die beste Behandlung zu finden, werden diskutiert, und zwar in bezug auf Faktoren wie Behandlungsresultat, Nebenerscheinungen, Spatkonsequenzen der Antibiotikabehandlung Nachweis bakterieller Genese und dem Vermogen verschiedener Antibiotika, die erkrankte Nasennebenhohle zu penetrieren

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ly for 10 days plus nasal decongestant, methazolinechloride (Nezeril®, Draco), three drops in each nostril three times daily for 10 days

Ampicillin + irrigation

Patients were treated with ampicillin as in group A and were irrigated every second day until the returned irrigation liquid was clear

Cephradine + nasal decongestant

Patients were treated orally with capsules of cephradine (Velosef®, Squibb) 0 25 g, four times daily for 10 days plus nasal decongestant as in group A

Cephradine + irrigation

Patients were treated with cephradine as in group C and were irrigated as in group B

Erythromycinestolate + nasal decongestant

Patients were treated orally with tablets of erythromycinestolate (Ilosone®, Lilly) 0 5 g three times daily for 10 days plus nasal decongestant as in group A

Erythromycinestolate + irrigation

Patients were treated with erythromycinestolate as in group E and were irrigated as in group B

The radiological healing was assessed on day five, ten and fifteen The radiological examination in the irrigation groups was always done before irrigation to reduce the risk of confusing the irrigation liquid with sinus secretion The radiological sinus changes were graded according to the following six-point scale below

No changes	0 points
Mucous membrane thickening 6 mm	1 point
Mucous membrane thickening >6 mm	2 points
Secretion	2 points
Mucous membrane thickening <6 mm + secretion	3 points
Mucous membrane thickening >6 mm + secretion	4 points
Complete opacity	6 points

The material was analysed by conventional methods including analysis of variance and covariance analysis The level of significance used in all tests was 95 % The material was analysed by computer technique, OSIRIS system, which besides the treatment result also assessed the concentration of antibiotic in sinus secretion and several other parameters The latter results will be reported separately

The present material comprises 299 patients with acute maxillary sinusitis analysed initially (Table I) In all 116 patients with 139 initially completely opaque maxillary sinuses, a single diagnostic irrigation was performed to demonstrate secretion The distribution of initially completely opaque sinuses in the six treatment groups is seen in Table I In 45 patients, the frontal sinuses were affected as well but these were not assessed during healing In 36 patients, the contralateral maxillary sinus demonstrated mucous membrane thickening but without secretion and these sinuses were also regarded In six contralateral sinuses without initial radiological changes, the subsequent radiological examination demonstrated a deterioration and these sinuses were included

RESULTS

There were no statistical differences in radiological states between the groups initially Further, the different treatment groups are comparable with respect to sex and age distribution as well as the number of unilateral and bilateral changes, the number of affected frontal sinuses and the number of completely and not completely opaque sinuses (Table I) All treatment groups demonstrated progressive healing during the treatment (Table II, Fig 1) There were no statistical differences in the radiological state between the groups on the fifth and fifteenth day The group treated with cephradine and nasal decongestant was statistically significantly less improved than the two groups treated with ampicillin + irrigation and erythromycinestolate + irrigation after 10 days' treatment (P 95) There was also a tendency for the cephradine + nasal decongestant group to have impro[illegible] than the other groups on the fifth a[illegible]

Table I *Acute maxillary sinusitis Present material Number of patients and diseased sinuses*

	Ampicillin nasal decon gestant	Ampicillin + irrigation	Cephradine + nasal decon gestant	Cephradine + irrigation	Erythromycin estolate + nasal decon gestant	Erythromycin estolate + irrigation	To
Women	33	31	33	30	32	32	19
Men	17	19	17	19	18	18	10
Total	50	50	50	49	50	50	29
Mean age years	33	34	34	36	31	36	20
Sinuses analysed initially	81	79	78	74	75	71	45
Sinuses analysed after 5 days	76	77	74	73	75	66	44
Sinuses analysed after 10 days	69	67	69	68	62	65	40
Sinuses analysed after 15 days	80	74	76	72	72	68	44
One sinus affected	18	17	20	20	22	27	12
Both sinuses affected	27	23	22	23	17	18	13
Frontal sinus also affected	5	10	8	6	11	5	4
Not completely opaque sinuses	56	58	58	46	58	43	31
Completely opaque sinuses	25	21	20	28	17	28	13

day The groups treated with irrigation were always more improved than those treated with nasal decongestant and the respective antibiotic There was a tendency for the groups treated with ampicillin + irrigation and erythromycin estolate + irrigation to be better radiologically than the other groups after 5 days of treatment

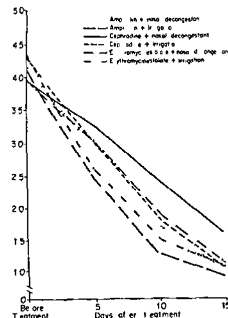
The side effects are noted in Table III In almost all instances they were mild and could often be attributed to the disease as well as to the treatment In two cases, the treatment with erythromycinestolate had to be stopped becau of side effects The most frequently report reactions were loose bowel movements and u specific fatigue

When the present material was divided in three groups with unilateral changes or bilater maxillary changes or bilateral maxillary ar frontal changes, there was initially a pro nounced difference between the groups Un lateral maxillary changes were more marked tha the other two On the fifth day, the unilater

Table II *Acute maxillary sinusitis Radiological gradation*[a] *of all maxillary sinuses before and duri treatment*

	Before treatment	Mean number of points sinus			% completely cured
		5 days	10 days	15 days	
Ampicillin + nasal decongestant	4 12	2 99	1 73	1 05	40
Ampicillin + irrigation	4 03	2.49	1 36	0 99	46
Cephradine + nasal decongestant	3 95	3 30	2 46	1 62	28
Cephradine + irrigation	4 26	2 99	1 85	1 13	33
Erythromycinestolate nasal decongestant	3 96	3 05	1 81	1 22	28
Erythromycinestolate irrigation	4 32	2 62	1 57	1 13	41

[a] The point scale adopted is given on page 467



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hanges had improved most The difference etween the groups was completely absent on e fifth, tenth, and fifteenth day This indicates hat apart from a rapid initial healing of uni ateral sinusitis, both unilateral and bilateral inusitis heal similarly during treatment and, urther, that the simultaneous involvement of he frontal sinuses does not prolong healing

DISCUSSION

The logical treatment of acute maxillary sinusitis, an infection situated in a cavity surrounded more or less by bone, should consist of restoration of drainage and antibacterial treatment Both of these therapeutical measures have well known advantages and disadvantages and/or it is by no means evident which method is preferable This in turn, is also due to a lack of appropriate methods with which to measure healing in sinusitis The patient s subjective impression, or the nasal status, or the appearance of the irrigation liquid are obviously too uncertain to give reliable information on the disease course

The ideal antibiotic would be the most effective one against bacteria most frequently demonstrated in the diseased sinus However, the knowledge available showing the bacterial flora in the aspirated sinus secretion has often shown conflicting firdings (Urdal & Berdal, 1949, Palva et al, 1962, Lystad, et al, 1964 Burdin et al, 1965, Kessler, 1968, Brandt et al, 1969, Krajina et al, 1969, Axelsson & Brorson, 1972, 1973*a*) Furthermore, in general nasal samples have been held to indicate the sinus flora which, however, does not reflect the sinus flora correctly (Axelsson & Brorson, 1973*a*) Even when aspirated sinus secretion is obtained

Table III *Side effects*

	Ampicillin + nasal decongestant	Ampicillin + irrigation	Cephradine + nasal decongestant	Cephradine + irrigation	Erythromycin estolate + nasal decongestant	Erythromycin estolate + irrigation
Fatique vertigo	8	7	6	8	8	7
Anorexia nausea vomiting		1	1	1	1	
Loose bowel movements[a] and/or abdominal pain	11	11	1	2	3	7
Intra hepatic cholestatic jaundice						1 (1)[b]
Deterioration of gastritis			1			
Obstipation		1			1	1
Epistaxis after irrigation		1		1		1
Leg cramps					1	1
Vaginal fluor and/or genital itching	1		2	1		1 (1)
Exanthema		1				
Number of patients	17	17	10	9	12	17

[a] More than one loose defecation on one or more days
[b] Figures within parentheses indicate the number of patients in whom treatment was interrupted because of side effects

for bacteriological examination, the sampling technique, the transport time and aerobic conditions influencing anaerobic bacteria unfavorably are some of the factors which might distort a correct bacteriological examination result This clearly demonstrates that bacteriological measurements are inappropriate for the measuring of healing in sinusitis

Another approach when choosing an antibiotic is comparison of the ability of different antibiotics to penetrate the sinus fluid Not many investigations have, however, as yet been performed (Lundberg et al, 1968, 1969, Gullers et al, 1969, Gnarpe & Lundberg, 1971, Lundberg & Malmborg, 1971, Jeppesen & Illum, 1972, Axelsson & Brorson, 1973*b*, 1974) Furthermore the concentration of antibiotics in sinus secretion gives no information on the course of healing

In the present investigation, the therapeutic outcome was assessed by sinus radiology during the course of healing This method was adopted because of the above described lack of other reliable, appropriate methods of measuring healing in sinusitis The advantages of the radiological examination are the reproducibility and the accuracy of the method in reflecting the stopathological sinus changes All the infectious anisms causing acute maxillary sinusitis in uce the same histopathological changes with mucous membrane thickening and secretion which can both be demonstrated by radiological examination

When dealing with the assessment of the cure of a disease, it is obviously important to define the disease concept In the present investigation, acute maxillary sinusitis is defined as radiological maxillary changes with secretion in patients with clinical symptoms indicating acute sinusitis Secretion was measured because it reflects a severe impairment of the sinus mucosa and the ostial function Allergic or vasomotor disorders might have been included in the present material also afflicting the sinus mucosa since they are difficult to exclude with certainty in the individual case Furthermore the secretion in the maxillary sinus of allergic or vasomotor origin is a favorable medium for growth of bacteria, even in cases which are primarily non infective, which is a common experience

The single initial diagnostic irrigation of the completely opaque maxillary sinuses to demonstrate secretion may have influenced healing The distribution, however, of completely opaque maxillary sinuses was fairly even among the groups

The result of the treatment in the present investigation shows that all groups healed similarly favorably except the group treated with cephradine+nasal decongestant This may be because the cephradine dosage was too low and because of low bone tissue concentrations (Neiss, 1973) To achieve a comparable treatment result with cephradine as with other therapeutic measures of the present and previous investigations, this antibiotic has to be combined with irrigation On the other hand, in treatment with ampicillin or erythromycinestolate the addition of irrigation did not further improve the healing course significantly The inferior result with cephradine was also reflected by comparatively low antibiotic concentrations in sinus secretion (Axelsson & Brorson, 1974) It appears from previous investigations that the most common antibiotics are effective against the most common agents causing acute maxillary sinusitis Consequently, in the choice between different antibiotics, other factors than the bacteriological finding and the bacterial resistance pattern must also be considered These include the occurrence and severity of side effects, the concentration of antibiotic in sinus secretion, the cost of treatment, etc From the patient's point of view, it is preferable to receive an antibiotic which is easy to take and requires fewer visits to the physician On the other hand, the widespread use and misuse of antibiotics must be diminished because of the increasing frequency of resistant strains, etc It can also be argued that the irrigation has less influence on the organism than has the antibiotic treatment One also has to consider the cost of therapy—in the one case, of antibiotic treatment with a higher medicine cost and in the other case, of irrigation with a higher treatment cost With

d to all these factors, it is difficult to choose optimal treatment

the present investigation, the best treatment ts were achieved, in order, by ampicillin, romycinestolate, and cephradine Contrari- ie frequency and severity of side effects was opposite Reports on the frequency and side ts in general show a great variety of results there are reasons for believing that the re- ed incidences of side effects in general are The reported frequency is dependent upon mode of questioning the patient, varying i a mere mentioning of side effects reported itaneously by the patient, to a detailed tionnaire of possible adverse effects in the vidual case In the present investigation, pa- s were asked about untoward reactions dur- and after completed treatment in the sense you experience any untoward reactions n the therapy All answers are included in le III In most cases, the side effects were rded as mild and the treatment did not need e stopped As expected, the most frequent tions were gastrointestinal The rather high urrence of fatique and vertigo can be ex- ned by the disease per se or by the antibiotic itment

e lack of controlled clinical studies of sinusi- is well known (Catlin et al, 1961) The anti- tics tested and compared in the present in- tigation are probably often used in clinical ctice and the literature contains some state- nts which generally reflect positive experien- with these antibiotics However, no con- lled studies in sinusitis have been found in the rature The different treatment modes tested d compared in the present controlled study n all be recommended, except cephradine + sal decongestant, which had inferior results to e other groups but relatively few side effects ie other five remedies achieved rapid healing th a moderate frequency of side effects It is vious that there is need for controlled studies f the "everyday diseases which from the ientific viewpoint seem unattractive but which ccording to their high frequency have important consequences

ZUSAMMENFASSUNG

Sechs Gruppen bestehend aus je 50 Patienten mit akuter Maxillarsinusitis wurden mit Ampicillin und Nasentropfen, Ampicillin und Kieferhohlenpunktion Cephadrine und Nasentropfen Cephadrine und Kieferhohlenpunktion, Erythromycinestolate und Nasentropfen sowie mit Erythromycinestolate und Kieferhohlenpunktion behandelt Die Diagnose wurde radiologisch verifiziert, und die Heilung wurde ebenfalls radiologisch kontrolliert, und zwar am funften, zehnten und funfzehnten Tag Die Behandlung erstreckte sich uber zehn Tage

Alle Gruppen zeigten radiologisch einen ahnlichen Heilungsverlauf mit Ausnahme der Gruppe, die mit Cephadrine und Nasentropfen behandelt wurde Diese zeigte weniger gute Resultate Dagegen waren Nebenerscheinungen bei Behandlung mit Cephadrine seltener und bei der Ampicillinbehandlung haufiger Die Schwierigkeiten, die beste Behandlung zu finden, werden diskutiert, und zwar in bezug auf Faktoren wie Behandlungsresultat, Nebenerscheinungen, Spatkonsequenzen der Antibiotikabehandlung, Nachweis bakterieller Genese und dem Vermogen verschiedener Antibiotika, die erkrankte Nasennebenhohle zu penetrieren

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